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Departamento de Biologia

Steven Ribeiro Alves

A relevância da sinalização da insulina na doença de Alzheimer.

The relevance of insulin signalling in Alzheimer's disease.

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A relevância da sinalização da insulina na doença de Alzheimer.

The relevance of insulin signalling in Alzheimer's disease.

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica da Professora Doutora Odete Abreu Beirão da Cruz e Silva, Professora Auxiliar com Agregação do Departamento de Ciências Médicas da Universidade de Aveiro.

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o júri

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Palavras-chave

Doença de Alzheimer, Sinalização da insulina, resistencia à insulina, Tipo 2 Diabetes, APP, Tau, fosforilação.

Resumo

A doença de Alzheimer (DA) é o tipo mais comum de demência no mundo. É caracterizada molecularmente pela deposição extracelular de placas senis (PS) compostas por agregados do péptido amiloide beta (Aβ), pela formação de emaranhados neurofibrilares (EN) derivados da hiperfosforilação da proteína Tau, pela disfunção sináptica devido aos depósitos de PS e EN e também pelo stress oxidativo induzido pelo enfraquecimento das vias metabólicas.

A via de sinalização da insulina desempenha um papel principal em diversas vias da DA, tal como na clivagem da APP, hiperfosforilação da proteína Tau, eficiência da sinalização da insulina influenciada pela Apolipoproteína E (ApoE) e pela enzima envolvida na degradação de insulina que também é a enzima principal na degradação de Aβ.

Crescente evidência relaciona a DA com a diabetes de tipo 2 (T2D) devido ao mau funcionamento da sinalização pela insulina e da resistência cerebral à mesma. Num estudo baseado num cohort da região de Aveiro, foi observada uma correlação entre a diabetes e um mau resultado no teste do 'Mini Mental State Examination'. Adicionalmente, também foi observada uma correlação entre os portadores do alelo ApoE-ε2 e um estado protetor contra a T2D. Este alelo também foi observado na literatura como sendo protetor contra a DA.

Posteriormente, uma análise de interações entre proteínas, identificou várias proteínas envolvidas tanto na DA como na sinalização da insulina. Correlacionando estes dados com o proteoma da sinapse, foi possível observar que existe uma grande representação das duas condições e também das proteínas coincidentes às duas (88% para a DA, 79% para a sinalização da insulina e 96% para as proteínas relacionadas com ambas), reforçando o papel de ambas as vias na sinalização e plasticidade sináptica. Do estudo de ontologia genética para a DA, foi possível identificar diversas vias importantes, tais como, resposta a um estímulo, organização de componentes celulares, comunicação celular, ligação proteica e ligação a uma cinase. Em relação à sinalização da insulina, as mesmas categorias apareciam com maior representação, significando que a insulina tem um papel importante em muitos eventos da DA.

Por fim, o tratamento de SH-SY5Y diferenciadas com 0, 1, 10 e 100 nM de insulina por 0, 10 e 60 minutos mostraram uma diminuição nos níveis intracelulares da proteína Tau e um aumento na sua fosforilação na serina 396. Em relação à proteína percursora amiloide (APP), o tratamento de insulina levou a um aumento nos níveis intracelulares, quando exposta por 10 minutos seguido por uma diminuição aos 60 minutos. Quanto à fosforilação da treonina 668 da APP, foi previamente demonstrado que um aumento na fosforilação desse resíduo, promove a clivagem pela via amiloidogénica, levando à produção de Aβ. Nas células tratadas com insulina, um aumento claro da fosforilação desse resíduo da APP foi observado aos 10 minutos. Aos 60 minutos, os níveis da fosforilação eram baixos provavelmente devido aos baixos níveis de APP total.

Keywords Alzheimer's disease, Insulin signalling, Insulin resistance, Type 2 Diabetes, APP, Tau, phosphorylation.

Abstract

Alzheimer's disease (AD) is the most common type of dementia worldwide. It is molecularly characterized by deposition of extracellular senile plaques (SPs) composed by aggregated amyloid beta (Aβ) peptide, the formation of neurofibrillary tangles (NFTs) derived from hyperphosphorylation of the microtubule-associated protein Tau, synaptic dysfunction due to the deposits of SPs and NFTs and oxidative stress induced by impaired metabolic pathways.

The insulin signalling pathway can play a major role in diverse AD related pathways, such as APP cleavage, Tau hyperphosphorylation, Apolipoprotein E (ApoE) influence in insulin signalling efficiency and the insulin degrading enzyme, which is also the major Aβ degrading enzyme.

Growing evidence links AD with type 2 diabetes (T2D) due to impaired insulin signalling (IS) and brain insulin resistance. In a cohort based study in the Aveiro region, a correlation between diabetes and poor cognitive scores in the Mini Mental State Examination (MMSE) test were observed, with a p-value of 0.072. Additionally, carriers of the allele ApoE-ε2 appeared to be protective against diabetes, in the literature the same allele appears to be protective for AD.

Posteriorly, the analysis of protein interactions, via the development of interactome networks, identified several proteins involved in both AD and the IS pathways. Also, by correlating these pathways with the synapse proteome, a very high overlap was observed (88% for AD, 79% for IS and 96% for AD and IS coincident proteins), enforcing the importance of both pathways in synaptic signalling and plasticity. From gene ontology studies, it was possible to assess the principal biological processes and molecular functions of the dataset of proteins. For AD, response to stimulus, cellular component organization, cell communication, signalling, protein binding, receptor binding and kinase binding were categories with elevated representation. Regarding coincident proteins between AD and IS pathways, an increase in all categories was observed, meaning that insulin plays a pivotal role in many AD events.

Finally, the analysis of SH-SY5Y differentiated cells treated with 0, 1, 10 and 100 nM of insulin for 0, 10 and 60 minutes, showed a decrease on the intracellular total levels of protein Tau and an increase in the phosphorylation at serine 396. Regarding the amyloid precursor protein (APP), increases in intracellular levels were observed, when treated with insulin for 10 minutes, followed by a decrease for 60 minutes exposure. The phosphorylation of APP at threonine 668, has previously been related to increased production of Aβ, by promoting APP cleavage via the amyloidogenic pathway. In cells treated with insulin, a clear increase was detected at the 10-minute time point. At 60 minutes, the levels of phosphorylation were low probably due to low total APP levels.

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III. Abbreviations

AD	Alzheimer's disease
ADL	Activities of Daily Living
AGE	Advanced glycation end-product
Akt/PKB	Protein kinase B
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Amyloid beta
BBB	Blood-brain barrier
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CASP3	Caspase-3
CDK	Cyclin-dependent kinase
CDR	Clinical Dementia Rating
ChAT	Choline acetyltransferase
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
ECL	Enhanced chemiluminescence
FBS	Fetal bovine serum
GDS	Global deterioration scale
GLUT4	Glucose transporter type 4
GO	Gene ontology
GRB2	Growth factor receptor-bound protein 2
GSK3	Glycogen synthase kinase 3
GSK3β	Glycogen synthase kinase 3 β
IADL	Instrumental activities of daily living
IDE	Insulin degrading enzyme
IR	Insulin receptor
IRS	Insulin receptor substrate
IS	Insulin signalling
IU	International units
LB	Loading buffer
LTP	Long-term potentiation
MAP1B	Microtubule-associated protein 1B
MAPK	Mitogen-activated protein kinase
MAPT	Microtubule-associated protein Tau
MEM	Minimum essential medium
MMSE	Mini–Mental State Examination
mTOR	Mechanistic target of rapamycin
NCSTN	Nicastrin
NFTs	Neurofibrillary tangles
PBS	Phosphate buffered saline
Pcb-cohort	Primary care based cohort
PDPK	Proline-Directed Protein Kinase
PHF	Paired helical fragments
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PP1	Protein phosphatase 1
PSEN1	Presenilin-1
PSEN2	Presenilin-2

RA	Retinoic acid
S	Serine
SAP	Stress-activated protein
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOS	Son of Sevenless
SPs	Senile plaques
T	Threonine
T2D	Type 2 diabetes
TBS-T	Tris-buffered saline-tween
Y	Tyrosine

1.Introduction

1. Introduction

1.1. Characterizing Alzheimer's disease

Alzheimer's disease (AD) is the most common type of dementia worldwide. It is estimated by the World Alzheimer Report of 2015 that 46.8 million people suffer from dementia and that this number will duplicate every 20 years.[1]

AD is a progressive disease characterized by a deterioration of memory and cognitive functions that can lead to death in 3 to 9 years after diagnosis.[2] In early stages of the disease, almost no symptoms occur, but in late-stages, there is complete loss of autonomy and no response to the environment. No cure is available for AD or other type of dementia, although some drugs can delay disease progression.[3]

Brain changes in AD can begin many years before symptom onset, but there is no efficient early diagnosis. Nevertheless, some possible risk factors have been described such as age, the presence of certain alleles of genes (particularly apolipoprotein E – ApoE-ε4)[45], gender (female)[6], low education levels[7], head trauma[8], small hippocampal volume[6], insulin resistance[9], high cholesterol, hypertension, reduced exercise and obesity[5].

The molecular mechanisms of the disease have been studied for many years and principal hallmarks have been well described. Amongst them the accumulation of extracellular senile plaques (SPs) caused by the deposition and aggregation of the peptide amyloid β (Aβ) which arises as a result of amyloid precursor protein (APP) cleavage[10,11]; the formation of neurofibrillary tangles (NFTs) derived from hyperphosphorylation of the microtubule-associated protein Tau[12,13] ; synaptic dysfunction due to the deposits of SPs and NFTs and the oxidative stress induced by impaired metabolic pathways[14].

1.1.1. Amyloid precursor protein cleavage and amyloid β production

The amyloid precursor protein (APP) is a transmembrane protein found in many tissues, and is targeted to the synapses of neurons. The precise function of APP is unknown, but some assumptions are possible. Mice with deficient APP show many phenotypes including reduced body and brain size, impaired learning and long-term potentiation (LTP) and hypersensitivity to seizures[15]. Thus it would appear that APP has an important role in memory associated events. At a cellular level, studies employing wild-type APP overexpression in transfected cell lines showed

improved cell growth, motility, neurite outgrowth and cell survival[16]. Thus APP, and APP derived fragments have been attributed with growth factor related characteristics.

APP processing is achieved by cleavage at specific sites and performed by specific proteolytic enzymes. APP can be cleaved by two different pathways, the amyloidogenic pathway and the non-amyloidogenic pathway. In the non-amyloidogenic pathway, APP is cleaved by α and γ-secretase, originating sAPPα and the peptides P3 and AICD (Figure 1). P3 is not toxic to the neurons and can be rapidly degraded[17]. On the other hand, in the amyloidogenic pathway, APP is cleaved by β and γ-secretase, originating sAPPβ and the peptides Aβ and AICD. The excess of Aβ, which aggregates as SPs, is one of the major contributors to the degeneration and dysfunction of neurons in AD[18].

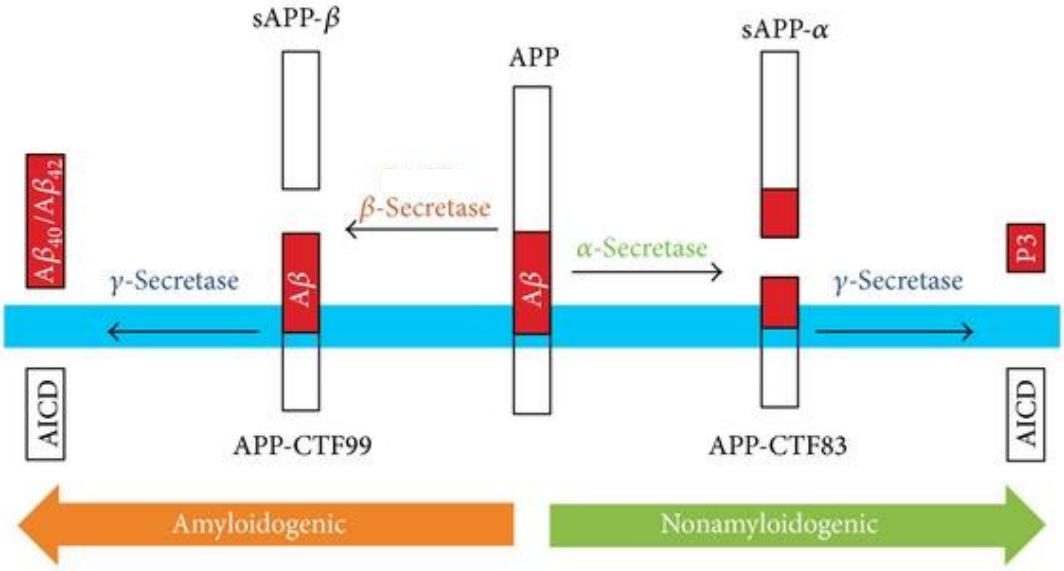

Figure 1-- APP cleavage via the amyloidogenic and non-amyloidogenic pathway. Secreted products obtained in the two pathways are indicated. Taken from: [19]

1.1.2. Hyperphosphorylation of microtubule-associated protein Tau

The protein Tau is expressed abundantly in the axons of neurons in the central nervous system, but can also be found in many others cells. This protein's most important role is to promote stability and assembly of microtubules, although this function can be complemented by other proteins, especially microtubule-associated protein 1B (MAP1B)[20], suggesting that Tau protein is not vital. Indeed, Tau knockout mice are viable, fertile, normal and show no signs of neurodegeneration. Complementing, knock out of Tau by small interfering RNA in primary neurons did not cause death, nor did it prevent axon formation and growth[21].

In AD, abnormal Tau phosphorylation and aggregation plays an important role in pathogenesis, forming intracellular neurofibrillary tangles (NFTs) that lead ultimately to cell death[22]. In the hyperphosphorylated state, Tau sequesters normal Tau and other proteins associated to microtubules, leading to microtubule destabilization and polymerization. Consequentially, axonal transport and neurotransmission are compromised, affecting cognitive functions. The major group of proteins involved in Tau phosphorylation are the proline-directed protein kinases (PDPK) that include glycogen synthase kinase 3 (GSK3), mitogen activated protein kinase (MAPK), Tau-tubulin kinase, cyclin-dependent kinase (principally CDK2 and CDK5) and stress-activated protein kinases (SAP kinases). In Tau extracted from AD patient brains, 45 phosphorylation sites (Figure 2) were discovered (29 serines (S), 13 threonines (T) and 3 tyrosines (Y)), of which, the majority can be phosphorylated by GSK3β, making this kinase, one of the most relevant in Tau hyperphosphorylation[13,23].

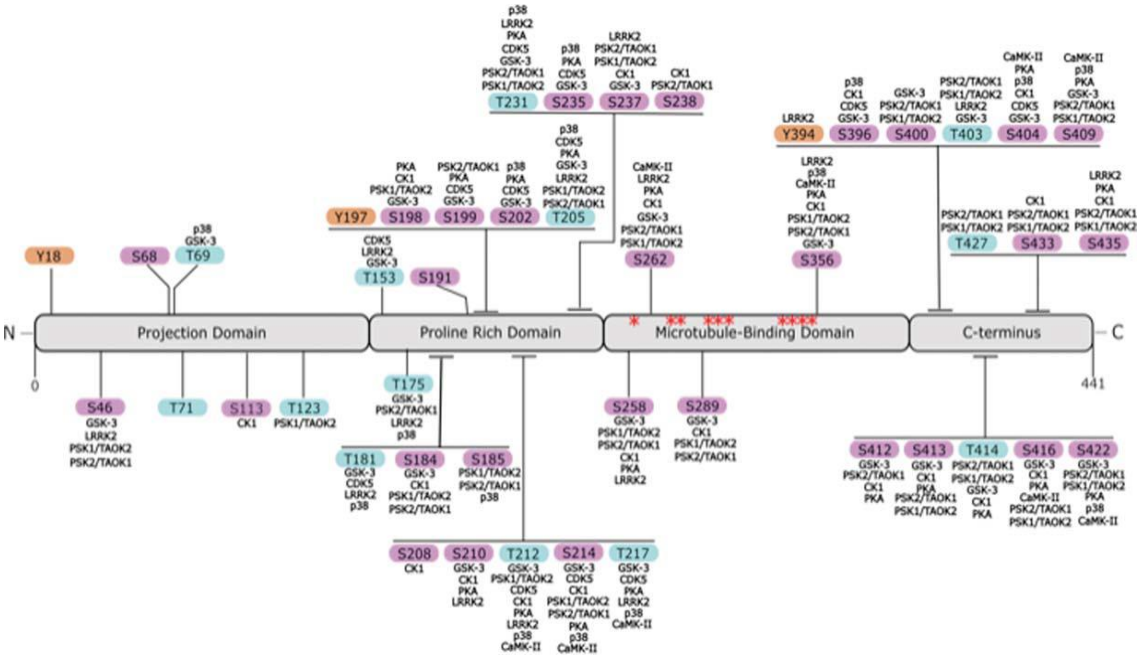

*Figure 2- Tau phosphorylation sites and responsible kinases retrieved from AD brains. S- Serine, T- Threonine and Y- Tyrosine. Microtubule – binding domain is indicated ***. Taken from:*[13]

1.2. Insulin signalling pathway

Insulin controls extensive biological processes by acting on a tyrosine kinase receptor. Receptor activation initiates a cascade of events involving phosphorylation, leading to activation or inactivation of many enzymes that control many physiological functions, namely metabolism and growth. Perturbations in the cascades can lead to insulin resistance[24].

Insulin's main actions are to stimulate the uptake of glucose and glycogen synthesis but it is also able to regulate many biological processes such as increased amino acid uptake, increased lipid synthesis, and decreased lipolysis, among others depending on the type of cells[25].

Many proteins are phosphorylated and dephosphorylated in the cascade of events when insulin binds to its receptor. Insulin and insulin receptor (IR) binding, results in a conformational change that induces the autophosphorylation of many tyrosine residues in the receptor[26]. The insulin receptor substrate family (IRS) recognizes these residues, which are, in turn, recognized by the phosphoinositide 3-kinase (PI3K). PI3K has the ability to phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and in the presence of phosphoinositide-dependent kinase-1 leads to the phosphorylation and activation of protein kinase B (PKB, also known as AKT). The activation of AKT is necessary for the regulation of many processes,

such as recruitment of Glucose transporter type 4 (GLUT4) to the membrane for glucose entrance, inhibition of GSK3α and β, resulting in glycogen synthesis and activation of the mammalian target of rapamycin (mTOR), which promotes protein synthesis[27](Figure 3). The binding of insulin to the IR, also triggers a second pathway, that leads to growth factor receptor-bound protein 2 (GRB2) activation, which in turn associates with the guanine nucleotide exchange factor son-of sevenless (SOS) leading to the activation of the mitogen-activated protein kinases (MAPK) pathway[28]. The activation of the MAPK pathway promotes cell division and differentiation.

It has only been a few years since the brain was considered as an insulin-sensitive organ. This was achieved by the detection of insulin and insulin receptors in the brain, although the origin of the insulin is still in debate. Its origin has been explained as deriving from peripheral or central sources, or even both. The discovery of insulin mRNA in the brain proved that it could be produced in the brain[29,30]. On the other hand, it was also shown that insulin can cross the blood-brain barrier (BBB) using a receptor mediated transport system[29–31]. Recent studies demonstrated that insulin regulates brain glucose and lipid metabolism, but it also regulates neuronal activities and development, playing an important role in learning and memory[30].

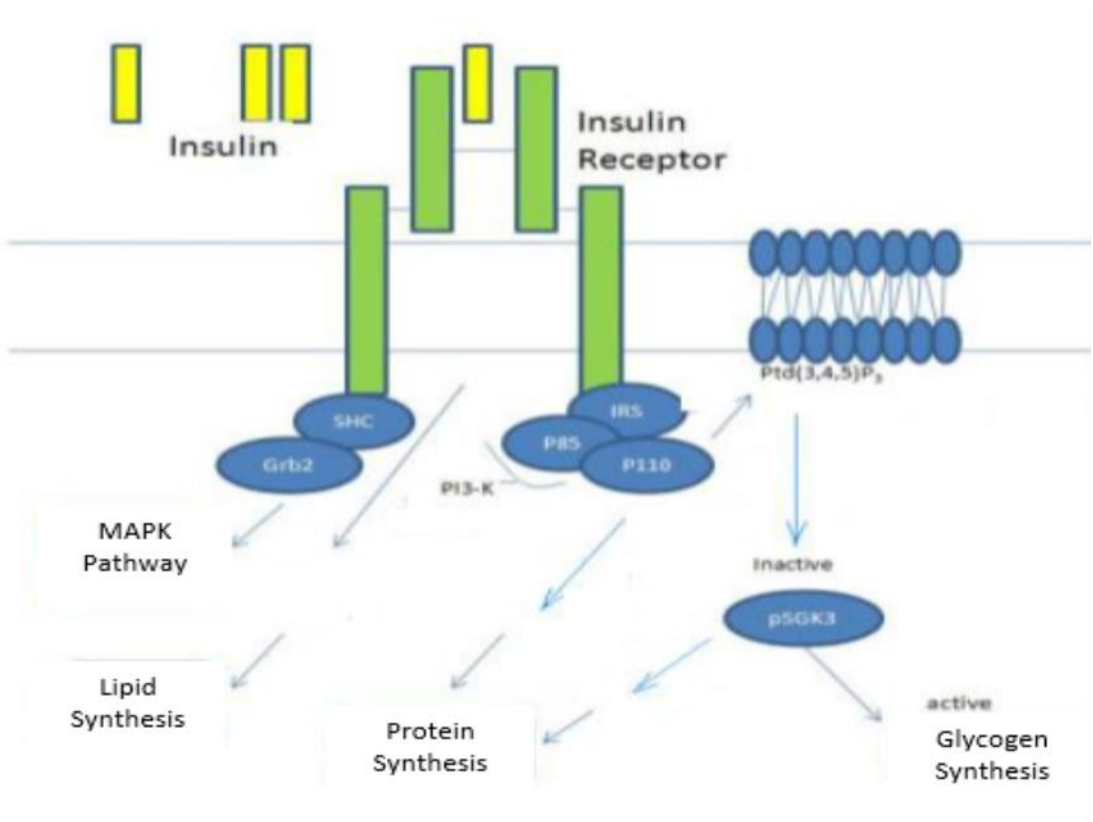

Figure 3- Different actions of insulin when bound to the receptor. Adapted from: [32]

1.3. Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2D) is a metabolic disorder that is characterized mainly by an elevation of glucose in the blood due to insulin resistance. This disease accounts for 90% of all cases of diabetes and is in exponential growth, as of 2014 records show that 422 million adults worldwide have T2D in comparison with only 108 million in 1980. Some people may be genetically predisposed to contract T2D[33], however this disease occurs mainly as a result of obesity and lack of exercise[34]. This condition is mainly controlled with the administration of metformin, leading to a decrease in glucose production by the liver and an increase in insulin sensitivity of body tissues[35].

Many factors can molecularly lead to insulin resistance. The most common include a decrease in the number of insulin receptors and of their catalytic activity, an increased Ser/Thr phosphorylation of the IR or the IRS, increase in tyrosine phosphatase activity, a decrease in PI3K and Akt kinase's activities and defective GLUT4 expression and/or function. These molecular changes lead to defective metabolism and a defective insulin cascade of events[36].

1.4. Insulin signalling pathway and Alzheimer's disease

In 1970 S. Hoyer reported reduced glucose levels and lower cerebral metabolic rates in the brains of demented individuals[37]. Today we know that many pathologies exhibit diminished brain glucose absorption, among them AD, Parkinson's disease, Multiple sclerosis, Huntington's Disease, Duchenne muscular dystrophy, forms of Autism, Down's Syndrome and Type I and Type II Diabetes Mellitus. The risk of developing AD is 50-60% greater in individuals with T2D[38]. Consistently, rats injected with streptozotocin intracerebroventricularly exhibit decreased glucose/energy brain metabolism and learning and memory deficits[39].

1.4.1. Insulin influence on APP cleavage

APP is one of the main proteins associated with AD. It is not known how the cell determines the pathways via which APP is processed. However insulin can play a major role in APP processing, promoting the non-amyloidogenic pathway. In a recent study, it was demonstrated that in the SH-SY5Y cell line insulin increased the levels of ADAM10 (α-secretase) and decreased those of BACE1 (β-secretase), leading to a decrease in Aβ production. Consistently, disturbances in the insulin cascade of events can be related with increases in Aβ production[40].

1.4.2. Tau phosphorylation by GSK3β

Glycogen synthase kinase-3 β (GSK3β) is a proline-directed serine/threonine kinases, expressed ubiquitously and involved in many cellular processes, namely glycogen metabolism[41], gene transcription[42], apoptosis[43] and microtubule stability[44,45].

This kinase is under close regulation of insulin and protein phosphatase 1 (PP1), both with the capacity to inactivate it. Phosphorylation of GSK3β at serine 9 by insulin turns the kinase inactive while PP1 has de ability to dephosphorylate the tyrosine 216 leading to the same inactive state[46].

In AD post mortem brain analysis, the values of PP1 appear diminished, leading to an increase activation of GSK3β. In the PC12 cell line, the presence of Aβ inhibited PP1[47], leading to hyperphosphorylated protein Tau, probably through dysregulation of GSK3β.

In T2D, deregulation in the insulin signalling (IS) pathway can also lead to GSK3β activation. Taken together, the deregulation of IS pathway and the inhibition of PP1 lead to a constant activation of this protein, continuously phosphorylating its substrates, namely protein Tau.

1.4.3. APOE effect on insulin efficiency

Apolipoprotein E (ApoE), in the brain, can be produced by both glia and neurons and its major role is to redistribute lipids among cells in the brain[48,49]. ApoE has 3 distinctive alleles (ApoE-ε2, ApoE-ε3 and ApoE-ε4), with ApoE-ε4 occurring in more than 50% of AD cases[49].

Clinical data showed that intranasal administration of insulin improved performance in learning and memory of elderly individuals. This performance is also correlated with the plasma levels of APP[50–52]. On the other hand, these effects are ApoE isoform dependent, where ApoE-ε4 carriers showed no improvement in memory tasks or no reduction of APP plasma levels[51,53].

A recent study in mice showed that insulin signalling impairment in the presence of Aβ was specific of ApoE genotype. The expression of ApoE-ε4 led to reduced insulin stimulation while ApoE-ε2 and 3 had preventive measures against Aβ, inducing proper insulin signalling[54].

These results are concurrent with another study that demonstrated that intranasal administration of 20 and 40 international units (IU) of insulin improved verbal memory of AD subjects, but only for those without the ApoE-ε4 allele[51].

1.4.4. The role of the insulin degrading enzyme (IDE) in AD

IDE, insulin degrading enzyme, is responsible for degrading insulin, however it is also the major enzyme responsible for degrading Aβ. Although IDE is elevated in AD brains, this does not translate into a higher Aβ clearance. On the contrary, IDE has more affinity for insulin than Aβ, which will lead to a higher degradation of insulin than normal causing a lower glucose metabolism. Hence insulin increases extracellular Aβ levels by promoting its secretion, but also by inhibiting its degradation via IDE. It is particularly noteworthy that IDE degrades Aβ in both microglial and neuronal cultures[55,56] and some APP isoforms are common to leucocytes and microglia, the LAPPs. Given that insulin resistance arises via a negative feedback loop of the insulin receptor pathway, this occurs under conditions of high insulin. The response involves the PI3J/AKT pathway (among others) as well as the insulin receptor substrates (IRSs). It is expected that induction of IDE will prevent the negative feedback and lower circulating insulin [57,58].

Many other cellular and molecular aspects are common to T2D and AD, these include Ca++ mediated signalling, formation of AGE (advanced glycation end-products), Wnt/β-catenin signalling, ChaT (ChAT, acetylcholine transferase) and JNK (c-Jun N-terminal kinase) mediated responses, these are beyond the scope of this project. It follows that the disease specific interactomes will unravel the involvement of several pathways, relevant to AD and T2D.

It would appear that a common pathophysiology in T2D and AD is becoming evident. Both conditions have a higher incidence with ageing and patients with T2D have a greater risk of developing AD. Hyperinsulinaemia and insulin resistance contribute to memory impairment and AD is increasingly perceived as also a metabolic disorder. The AD brain loses the capacity to: utilize glucose efficiently; respond to critical trophic factor signals and develops resistance to insulin and insulin growth factor (IGF). Glucose is the main brain energy source; these anomalies cause the brain to 'starve' accounting for AD associated abnormalities. Thus the term Type 3 diabetes (T3D) has been coined for AD. In closing, individuals with T2D have a higher risk of developing AD, but the cellular mechanisms remain unknown. Of concern diabetes medication could increase dementia risk. Metformin, prescribed for T2D, increases BACE1 levels and Aβ production, propagating AD progression. On a more positive note, insulin in combination with metformin may override the BACE1 effect and be beneficial to T2D+AD (T3D) patients[59]. Thus, it is fundamental to unravel the underlying cellular signalling cascades that can contribute to patient management and well-being.

2. Objectives of the study

2. Objectives

The main goal of this work is to determine if a correlation between Alzheimer's disease and type 2 diabetes at a clinical level, exists. For this purpose, it is necessary to explore different tools in order to unravel molecular mechanisms behind the two conditions, such as statistical population studies, bioinformatics and also molecular mechanisms.

Thus the specific objectives are to:

- Address if there is an association between cognitive capacities and diabetes;
- Unravel interactome networks relevant to AD and Insulin signalling;
- Optimize conditions for differentiating a neuronal-like cell line;
- Determine the effect of insulin on AD relevant molecules.

3. Materials and methods

3. Materials and methods

3.1. Epidemiological observation

A cross-sectional population-based survey was previously carried out on a Portuguese population, in the Aveiro district[60]. Volunteers were admitted, independent of complaints or deficits in any cognitive domain. A mandatory inclusion criteria to be 50 years or older was observed. Individuals undergoing oncological treatment, diagnosed with psychiatric disorders (excluding depression), aphasia, or unable to answer the questions in the structured interview were excluded. To ensure generalizability, exclusion criteria were kept to an absolute minimum. Participants underwent a semi-structured questionnaire addressing sociodemographic characteristics, personal and family history. Subsequently questions typically associated with diagnosing dementia were asked. Clinical history and medications taken were collected from clinical records and registered. The study group was designated the primary care based cohort, pcb-Cohort.

Figure 4- Workflow for the study design.

Cognitive evaluations and dementia screening tests, irrespective of the clinical diagnosis, were performed during the interview. These included Clinical Dementia Rating (CDR)[61], Mini Mental State Examination (MMSE), Geriatric Depression Scale (GDS)[62], Katz of Activities of Daily Living (ADL)[63] and Instrumental Activities of Daily Living (IADL)[64]. The tests CDR, MMSE, GDS and IADL had been previously standardize for the Portuguese population[65].
Participants were also scored for comorbidities, including diabetes. Volunteers were recruited in primary health centers (pcb-Cohort) in the Aveiro region.

3.2. Network construction

The protein networks were built based on protein-protein interactions that are found on the available literature, relevant to Alzheimer's disease (AD) and Insulin signalling (IS) pathways. The first step was to determine the key proteins (table 1 and 2) in order to build the interactome for each of these proteins. These key proteins were determined using KEGG pathway, for both pathways.

Table 1- Alzheimer's disease pathway key proteins

Gene name	Uniprot code	Protein complete name
APOE	P02649	Apolipoprotein E
APP	P05067	Amyloid beta A4 protein
BACE1	P56817	Beta-secretase 1
MAPT / TAU	P10636	Microtubule-associated protein tau
PSEN1	P49768	Presenilin-1
PSEN2	P49810	Presenilin-2
ADAM10	O14672	Disintegrin and metalloproteinase domain-containing protein 10
ADAM17	P78536	Disintegrin and metalloproteinase domain-containing protein 17
APH1A	Q96BI3	Gamma-secretase subunit APH-1A (Presenilin-stabilization factor)
APH1B	Q8WW43	Gamma-secretase subunit APH-1B
CAPN1	P07384	Calpain-1 catalytic subunit
CAPN2	P17655	Calpain-2 catalytic subunit
CASP3	P42574	Caspase-3
CASP7	P55210	Caspase-7
CDK5	Q00535	Cyclin-dependent-like kinase 5
CDK5R1	Q15078	Cyclin-dependent kinase 5 activator 1
IDE	P14735	Insulin-degrading enzyme
LPL	P06858	Lipoprotein lipase
LRP1	Q07954	Prolow-density lipoprotein receptor-related protein 1
MME/NEP	P08473	Neprilysin
NCSTN	Q92542	Nicastrin
PSENEN	Q9NZ42	Gamma-secretase subunit PEN-2
RTN3	O95197	Reticulon-3
RTN4	Q9NQC3	Reticulon-4

Table 2-Insulin signalling pathway key proteins

Gene name	Uniprot code	Protein complete name
AKT1	P31749	RAC-alpha serine/threonine-protein kinase
AKT2	P31751	RAC-beta serine/threonine-protein kinase
AKT3	Q9Y243	RAC-gamma serine/threonine-protein kinase
GLUT4/SLC2A4	P14672	Solute carrier family 2, facilitated glucose transporter member 4
GRB2	P62993	Growth factor receptor-bound protein 2
GSK3A	P49840	Glycogen synthase kinase-3 alpha
GSK3B	P49841	Glycogen synthase kinase-3 beta
INSR	P06213	Insulin receptor
IRS1	P35568	Insulin receptor substrate 1
IRS2	Q9Y4H2	Insulin receptor substrate 2
MAPK1	P28482	Mitogen-activated protein kinase 1
MAPK3	P27361	Mitogen-activated protein kinase 3
PIK3CA	P42336	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
PIK3CB	P42338	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform
PIK3CD	O00329	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform
PIK3R1	P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha
PIK3R2	O00459	Phosphatidylinositol 3-kinase regulatory subunit beta
PIK3R3	Q92569	Phosphatidylinositol 3-kinase regulatory subunit gamma
PDPK1	O15530	3-phosphoinositide-dependent protein kinase 1
SOS1	Q07889	Son of sevenless homolog 1
SOS2	Q07890	Son of sevenless homolog 2
HRAS	P01112	GTPase HRas
KRAS	P01116	GTPase KRas
NRAS	P01111	GTPase NRas
ARAF	P10398	Serine/threonine-protein kinase A-Raf
RAF1	P04049	RAF proto-oncogene serine/threonine-protein kinase
BRAF	P15056	Serine/threonine-protein kinase B-raf
MAP2K1	Q02750	Dual specificity mitogen-activated protein kinase kinase 1
MAP2K2	P36507	Dual specificity mitogen-activated protein kinase kinase 2
MTOR	P42345	Serine/threonine-protein kinase mTOR
BAD	Q92934	Bcl2-associated agonist of cell death
FOXO1	Q12778	Forkhead box protein O1

Posteriorly, the uniprot code for the *Homo sapiens* of each of the key proteins was retrieved (Table 1 and 2), in order to eliminate interactions described in other species, and were submitted into the IntAct Molecular Interaction Database. Different databases (IntAct, String and BioGRID) were tested and IntAct demonstrated to be more complete and reliable, as well as including only curated data.

All data recovered from IntAct were imported to Cytoscape and the interaction networks were built for each protein.

In order to get the interactome for the AD pathway and the IS pathway, the interactomes of each protein from each pathway were merged and organized for better visualization. Also, the list of all protein interactions of each pathway were crossed so as to identify coincident proteins. These were marked in a different colour in the network.

Also a network for the synapse was built. All synaptic proteins were obtained by a Gene Ontology (GO_0045202) predefined list already assembled in the literature.

3.3. Neuronal cell models

The use of human in vitro neuronal cell models has been fundamental to understand the functioning of the nervous system. Cell models play an important role in reducing animal testing and provide a better understanding of human cells, because of the identical genome.

The culture of neuronal cells is hard to achieve, since mature neurons do not undergo significant cell division. For this purpose, it is necessary to use cell lines that are derived from neuronal tumours and have become immortalized. These cells are easier to grow and present small variability between cultures.

3.3.1. SH-SY5Y cell line

SH-SY5Y (ATCC® CRL-2266™) cell line are a subclone of the neuroblastoma cell line SK-N-SH. This cell line was generated from a bone marrow biopsy in 1970. It is composed of two types of cells: neuroblast-like and epithelial-like. The SH-SY5Y cell line can be differentiated into a neuronal mature human state through different mechanisms, depending on the neuron subtype required (adrenergic, cholinergic or dopaminergic). This cell line has been extensively used as a neuronal cell model due to the resemblance to primary neurons.

Both differentiated and non-differentiated SH-SY5Y cells are used in in-vitro experiments, depending on the target in study. Undifferentiated cells tend to grow in clusters and are morphologically neuroblast-like with non-polarized cell bodies. They also lack mature neuronal

markers. In contrast, differentiation into neuronal-like cells leads to different events, including the induction of neuron-specific enzymes, neurotransmitter and neurotransmitter receptors, formation and extension of neuritic processes, synaptophysin-positive functional synapses and increased electrical excitability of the plasma membrane. Thus for this reason we opted to differentiate the SH-SY5Y cell line and use it in subsequent experiments.

3.2.2. SH-SY5Y cell culture

3.2.2.1. Growth and maintenance of SH-SY5Y cell line

The complete growth medium used to maintain this cell line is composed of a 1:1 mixture of Eagle's Minimum Essential Medium (MEM) and F12 supplemented with 10% of heat-inactivated Fetal Bovine Serum (FBS), 2nM L-Glutamine, sodium bicarbonate, sodium pyruvate and 1% of antibiotic/antimycotic.

Prior to use, cells were cryopreserved in liquid nitrogen at -196ºC with a cell freezing medium composed by complete growth medium supplemented with 5% (v/v) dimethyl sulfoxide (DMSO).

To use, cells were quickly thawed to 37ºC, 1ml of cell media was added to the vial and centrifuged at 1000 rpm for 3 minutes to remove the DMSO. The pellet of cells was re-suspended in fresh complete growth medium and seeded in a 100mm culture plate.

Cell cultures were maintained in an incubator at 37ºC with 5% CO_2 and 95% humidity. Every three days, complete growth medium was replaced until cell splitting. To split the cells, all medium was aspirated and cells were washed with 2 ml of pre-warmed Phosphate Buffered Saline (PBS). Afterwards, the PBS was aspirated and 2ml of 0,05% of Trypsin-EDTA solution was added to re-suspend adherent cells. After cell detachment, 6ml of fresh complete growth medium was added to inactivate the trypsin, followed by centrifugation at 1000 rpm for 3 minutes in a 15ml vial. Finally, the supernatant was carefully discarded, cells were re-suspended in fresh medium and plated at the desired cell density.

3.2.2.2. Differentiation of SH-SY5Y cell line

The differentiation of this cell line is mainly achieved by the addition of Retinoic Acid (RA) to the complete growth medium.

The literature offers many options for the differentiation of the SH-SY5Y cell line, a protocol optimization was necessary to test the optimal conditions. For this, the most important variables on the protocols was the percentage of fetal bovine serum (FBS) used and the time of exposure to the RA. Four hundred thousand cells were plated in each well of a 6-well plate (diameter 34.8 mm) and two concentrations of FBS (1% and 10%) were tested in combination with 10 µM of RA. Phase-contrast photographs were taken at day 0, 3 and 5, and cell elongation and neurites outgrowth were assessed.

3.2.2.3. Insulin treatment

Human insulin was obtained from Sigma-Aldrich (product code I3536) and reconstituted in PBS. To test the influence of insulin, cells were exposed to 0, 1, 10 and 100 nM of insulin in combination with serum free medium for 0, 10 and 60 minutes at 37ºC.

3.3. Sample preparation and protein quantification

After treatments, cells were washed with ice-cold PBS and then collected using RIPA lysis buffer (Sigma, cat. #R0278) and a cocktail with protease and phosphatase inhibitor (cOmplete, EDTA-free, Roche, cat. #11873580001). Subsequently, lysates were sonicated during 10 seconds and stored at -20ºC.

Protein quantification was obtained using bicinchoninic acid (BCA) assay, using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, cat. #23225). This assay relies on two different reactions. Initially, the ability of peptide bonds to reduce Cu^{2+} to Cu^{+}. The amount of Cu^{2+} reduced is proportional to the amount of protein in the sample. This reaction is followed by the chelation of

molecules of BCA to one molecule of Cu^{+} forming a purple-coloured complex exhibiting a strong absorbance at 562 nm.

In order to determine the precise amount of protein in the sample, it is necessary to use a standard where the amount of protein is previously known as described in the table 3.

Table 3- Standards used in BCA. Bovine Serum Albumin (BSA).

Standard	BSA (µL)	Buffer (µL)	Protein mass (µg)
P0	-	25	0
P1	1	24	2
P2	2	23	4
P3	5	20	10
P4	10	15	20
P5	20	5	40

For protein quantification, 5 µl of SH-SY5Y lysates were used. Standards and samples were incubated with 200 µl of working reagent previously prepared by adding 50 parts of reagent A and 1 part of reagent B from the Pierce™ BCA Protein Assay kit. The plate was incubated for 30 minutes at 37ºC and the absorbance was immediately measured at 562nm using TECAN Infinite M200.

3.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is used to separate proteins from a mixture based on molecular weight. This technique relies on the capacity of proteins to migrate through gel pores under an electrical field. SDS needs to be used in this method to remove different protein charges and in order to charge negatively all proteins, enabling the sample to migrate from a negative to a positive pole. A reducing agent (β-mercaptoethanol) is also used in combination with SDS to dissociate proteins before loading the gel. This ensures that all disulphide bonds are cleaved, linearizing the proteins so that the tertiary and quaternary protein structures do not interfere with the migration in the gel.

In this work, samples were subjected to 5-20% gradient SDS-PAGE in a Hoefer electrophoresis system. The gel is composed of 2 phases, the staking gel, where the samples are loaded, and the resolving gel, where the proteins are separated. The resolving gel has a linear progression in the concentration of acrylamide from top to bottom, in order to get a wider separation range.

Firstly, the resolving gel solution was prepared and carefully pipetted into the mounted electrophoresis equipment and distilled water added on top to reduce toxicity to the environment. The gel was left for 1h at room temperature in order to polymerize. Subsequently, the stacking gel solution was prepared, the water from the equipment was poured out and the solution loaded on top of the running gradient gel. A comb was inserted to mark the wells for the samples and the gel was left a further 1 hour to polymerize at room temperature.

In parallel, samples were prepared by adding to the protein sample solution, ¼ volume of 4X loading buffer (LB) and boiled for 10 minutes. The samples and the molecular weight marker (Precision Plus ProteinTM Dual Color Standards, Bio Rad, cat. #1610374) were then carefully loaded into the wells and separated by electrophoresis at 90 mA of electric current for 3 to 4 hours.

3.5. Western blot

Western blot or Immunoblot is a widely used technique for detection and analysis of proteins. This technique is used to detect specific proteins in complex samples like cell lysates or body fluids. Following the SDS-PAGE, the proteins are transferred to a solid membrane, in this case, a nitrocellulose membrane, by an electrophoretic field while the proteins positions remain unaltered. Once in the membrane, proteins are suitable for detection by protein staining or labelling by specific antibodies. The membranes can be stained with Ponceau S, to confirm protein transfer and assess equal gel loading. Before incubating with antibodies, the membranes need to be blocked with either non-fat milk or BSA, in order to block non-specific sites of the primary antibody. Membranes are then incubated with the specific primary antibody followed by the secondary antibody. After the binding, the antibodies can be detected by chemiluminescence and detected using appropriate equipment.

Transfer was confirmed by Ponceau staining. Ponceau S is a dye that binds to protein positive amino acids and to non-polar protein regions in a non-covalent form. For this purpose, the

membrane was hydrated with Tris-buffered saline solution (TBS) during 10 minutes under agitation, followed by the ponceau staining solution for 5 minutes. After the incubation, the solution was removed from the membrane using deionized H_2O to remove the excess.

After confirming the transfer and totally removing ponceau staining, membranes were blocked with 5% BSA in tris buffered saline tween (TBS-T) 1x for 4 hours. The membranes were subsequently washed in TBS-T 1x for 10 minutes and incubated with a specific primary antibody overnight. In the next morning, membranes were washed six times with TBS-T 1x for 10 minutes each and incubated with the appropriated secondary antibody for 2h. Following the incubation, the membranes were washed six times with TBS-T 1x for 10 minutes each and incubated with enhanced luminescence (ECL) detection kit for 5 minute, followed by visualization of the membrane using ChemiDoc Imaging System.

3.6. Resazurin assay

Resazurin is a cell permeable redox indicator that can be used to monitor cell viability. Resazurin is a blue dye that can be dissolved in physiological buffers or cell culture medium. Cells with active metabolism can reduce resazurin into resorufin, which is pink and fluorescent. The quantity of resorufin that is produced is proportional to the number of viable cells which can be quantified using a microplate equipped with fluorimeter with a 560 nm excitation and 590 nm emission filter set.

For this assay, differentiated SH-SY5Y cells were pre-treated with different concentrations of insulin (0, 1, 10 and 100 nM) for 0, 10 and 60 minutes, followed by incubation with 10 µg/ml of resazurin for 4 hours.

4. Results and Discussion

4. Results and discussion

4.1. Diabetes correlates with poor cognitive scores

The MMSE was applied to the pcb-Cohort and scored from 0-30. Cut-off was set at ≥7 years of literacy and MMSE cut-off at 27 [66]. Those equal to or below the cut-off are scored as having cognitive deficit, those above the cut-off as normal. Individuals were also analyzed with respect to comorbidities (Table 4). A positive correlation with neuropathological disorders was obtained for the three MMSE cut-off analyses (Table 4). In effect one can confirm that the diagnosis of neuropathological conditions was correct in this population. With respect to other comorbidities the strongest correlation was obtained with diabetes (DM) and MMSE 27 with a p-value of 0.072 (Table 4).

Table 4- Association of cognitive performance with comorbidities. Taken from:[67]

Clinical Characteristic	N	MMSE Cut Point 27		
		Negative (N=436)	Positive (N=132)	p-value
Number of Disease	-	2.98 ± 1.68 [a]	3.11 ± 1.68 [a]	0.466
Hyp*	351	265 [a] (60.8%)	86 [a] (65.2%)	0.365
Dys*	333	257 [a] (58.9%)	76 [a] (57.6%)	0.780
OA*	305	227 [a] (52.1%)	78 [a] (59.1%)	0.156
CVD*	302	234 [a] (53.7%)	68 [a] (51.5%)	0.664
Dep*	182	136 [a] (31.2%)	46 [a] (34.8%)	0.430
GID*	151	112 [a] (25.7%)	39 [a] (29.5%)	0.379
GUD*	122	98 [a] (22.5%)	24 [a] (18.2%)	0.292
DM*	115	81 [a] (18.6%)	34 [a] (25.8%)	**0.072**
Resp*	93	70 [a] (16.1%)	23 [a] (17.4%)	0.710
Hemato*	60	46 [a] (10.6%)	14 [a] (10.6%)	0.985
Onco*	32	26 [a] (6.0%)	6 [a] (4.5%)	0.536
Neuro*	22	9 [a] (2.1%)	13 [b] (9.8%) [b]	**<0.001**
Alc*	14	9 [a] (2.1%)	5 [a] (3.8%) [a]	0.263

Additionally the correlation of diabetes with other factors was also evaluated. The CDR applied a 0 to 3 score [61], where 0=Normal, 0.5=suspect, questionable or very mild dementia, and CDR≥1(1, 2, 3)=mild, moderate or severe dementia. The reduced GDS was also applied, version GDS-15 [62]. Results were scored from 0-15, where 0-4 is normal and above 5 is consistent with depression. IADL was applied to evaluate eight daily functionalities [64]. Results are shown in table 5.

Table 5- Association of diabetes with cognitive evaluation and ApoE genotyping

Characteristics	N(%)	Diabetes Mellitus		Statistical test	*p-value*
		No N= 453	Yes N= 115		
Cognitive Evaluation					
MMSE27	**132 (23,2%)**	**98 (21,6%)**	**34 (29,6%)**	**χ^2= 3.325**	**0.072**
CDR ≥ 1 +MMSE27	**15 (47,0%)**	**42 (48,1%)**	**9 (42,6%)**	**χ^2= 9.918**	**0.078**
CDR ≥ 0.5	267 (47,0%)	218 (48,1%)	49 (42,6%)	χ^2= 1.120	0.290
GDS	174 (30,6%)	137 (30,2%)	37 (32,2%)	χ^2= 0.161	0.688
IADL	176 (31,0%)	137 (30,2%)	39 (33,9%)	χ^2= 0.578	0.477
APOE *Genotyping*					
ApoE ε2 carriers	**38 (7.5%)**	**35 (8.6%)**	**3 (3.0%)**	**χ^2=3.611**	**0.057**
ApoE ε3 carriers	494 (97.2%)	396 (97.1%)	98 (98.0%)	χ^2=0.265	0.606
ApoE ε4 carriers	96(18.9%)	79 (19.4%)	17(17.0%)	χ^2=0.293	0.589

A strong correlation between poor cognitive performance and diabetes was again observed (table 5) with a p-value of 0.072 for MMSE27 and p-value of 0.078 for CDR and MMSE positive individuals. Additionally it is also clear that the ApoE-ε2 genotype (p-value of 0.057) is protective for diabetes, of note and as described in the introduction, it is also protective for AD.

The data here presented provides strong preliminary evidence linking poor cognitive scores to diabetes and validates the need to carry out further molecular and cell biology based studies.

4.2. Network analysis

4.2.1. Key proteins interactomes

In order to understand the interrelationship of proteins we are increasingly resorting to mapping complex networks using a systems biology approach. Often the central point (protein/node) is a protein whose involvement has been well characterized for a given pathology; such as the APP in AD. Hence using the list of proteins in Table 1 and 2, see methods above, the interactome for each of the proteins involved in AD and IS was constructed. In essence a network was built for every key protein in both AD and IS pathway. An example is shown in Figure 5.

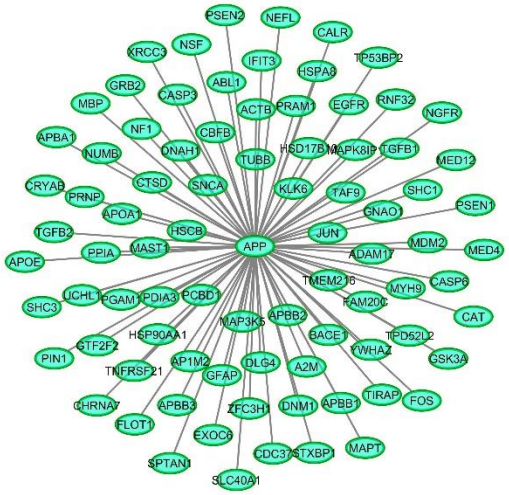

Figure 5 - APP interactome, as example.

To simplify the data output the number of interacting proteins with each of the search nodes/proteins is shown in Table 6 and 7. Of note, the IntAct database identifies the gene but given that we are discussing proteins the term protein will be used.

Table 6 - AD proteins and the number of interactors involved

	Gene name	Number of interactors
AD	APOE	25
	APP	84
	BACE1	5
	MAPT / TAU	28
	PSEN1	36
	PSEN2	21
	ADAM10	4
	ADAM17	8
	APH1A	14
	APH1B	1
	CAPN1	18
	CAPN2	11
	CASP3	40
	CASP7	12
	CDK5	58
	CDK5R1	7
	IDE	7
	LPL	14
	LRP1	17
	MME/NEP	81
	NCSTN	49
	PSENEN	7
	RTN3	20
	RTN4	38

Table 7- IS proteins and the number of interactors involved

	Gene name	Number of interactors
IS	AKT1	144
	AKT2	31
	AKT3	7
	GLUT4/SLC2A4	3
	GRB2	760
	GSK3A	28
	GSK3B	199
	INSR	35
	IRS1	23
	IRS2	15
	MAPK1	87
	MAPK3	88
	PIK3CA	38
	PIK3CB	13
	PIK3CD	9

	PIK3R1	186
	PIK3R2	50
	PIK3R3	123
	PDPK1	49
	SOS1	31
	SOS2	15
	HRAS	42
	KRAS	56
	NRAS	30
	ARAF	80
	RAF1	106
	BRAF	39
	MAP2K1	60
	MAP2K2	17
	MTOR	64
	BAD	34
	FOXO1	33

Consequently all the interacting networks for AD were merged, the same was carried out for the IS. Highly complex networks were obtained.

Obviously it is difficult to simultaneously address the functional significance of all the proteins/nodes present in the merged networks. Hence, a subsequent analysis was carried out to identify, which of the proteins present in the AD and IS pathways were also relevant to the synapse.

4.2.2. Synapse network correlation

A synapse proteome network including 6900 proteins was created (data not shown) to correlate the presence of AD proteins in the synapse. This is singularly important as in AD synapses are destroyed, causing loss of communication between neurons and leading, in a final stage, to neuronal death. For this purpose, the 511 proteins retrieved from the merged network of key AD proteins, were crossed with the 6900 synapse proteins found in the literature. It was possible to identify that AD and the synapse are related, as 88% of the retrieved AD pathway proteins can be found in the synapse proteome (Supplementary table 1).

The same procedure was carried out for the IS pathway, in order to see the relevance of IS in the synapse. Although the number of IS proteins retrieved is much higher, 79% of these were present in the synapse which highlights the relevance of IS pathway to the synapse (Supplementary table 1).

If one considers the overlap between merged networks for the AD and IS pathways there are 156 shared nodes/proteins, these are discussed in more detail below, but of these 150 are also present in the synapse (Supplementary table 1). In other words 96% of AD and IS shared proteins can be found in the synapse, re-enforcing that the IS pathway in the synapse may be of critical importance to AD.

Table 8- Number and percentage of proteins involved in each pathway and in the synapse.

Pathway	Number of proteins retrieved from the disease	Number of proteins coincident with the synapse	Percentage of coincident proteins with the synapse
AD pathway	511	449	88%
IS pathway	1728	1358	79%
Coincident in AD and IS pathway	156	150	96%

4.2.3. Alzheimer's disease and insulin signaling pathway correlation

As mentioned above, 156 proteins/nodes were identified as coincident in AD and the IS pathways, but further analysis was required. The network of all AD retrieved proteins served as a template and all 156 proteins (Table 7) were marked to help visualize common proteins and their probable functions in AD (Figure 6 and 7).

Table 9- Proteins identified as coincident in AD and the IS pathway.

Gene names	Proteins full name
A2M	Alpha-2-macroglobulin
ABL1	Tyrosine-protein kinase ABL1
ACTB	Actin, cytoplasmic 1
ACTN1	Alpha-actinin-1
AKT1	RAC-alpha serine/threonine-protein kinase
AKT2	RAC-beta serine/threonine-protein kinase
AKT3	RAC-gamma serine/threonine-protein kinase
ALAS1	5-aminolevulinate synthase, nonspecific, mitochondrial
ANXA6	Annexin A6
APBB3	Amyloid-beta A4 precursor protein-binding family B member 3
APOA1	Apolipoprotein A-I
APOB	Apolipoprotein B-100
APP	Amyloid-beta A4 protein
AR	Androgen receptor
ARRB2	Beta-arrestin-2
ASCC2	Activating signal cointegrator 1 complex subunit 2
ATF2	Cyclic AMP-dependent transcription factor ATF-2
ATP5A1	ATP synthase subunit alpha, mitochondrial
BCAR3	Breast cancer anti-estrogen resistance protein 3
BCL2L1	Bcl-2-like protein 1
BIN1	Myc box-dependent-interacting protein 1
CALR	Calreticulin
CASP3	Caspase-3
CAST	Calpastatin
CCNB1	G2/mitotic-specific cyclin-B1
CCND1	G1/S-specific cyclin-D1
CCNI	Cyclin-I
CCT3	T-complex protein 1 subunit gamma
CCT7	T-complex protein 1 subunit eta
CCT8	T-complex protein 1 subunit theta
CDC37	Hsp90 co-chaperone Cdc37
CDC42	Cell division control protein 42 homolog
CDH1	Cadherin-1
CDKN1A	Cyclin-dependent kinase inhibitor 1
CDKN1B	Cyclin-dependent kinase inhibitor 1B
CEP126	Centrosomal protein of 126 kDa
CFL1	Cofilin-1
COPS6	COP9 signalosome complex subunit 6
CREB3	Cyclic AMP-responsive element-binding protein 3
CRELD2	Cysteine-rich with EGF-like domain protein 2
CTNNB1	Catenin beta-1
CTSD	Cathepsin D
CTTN	Src substrate cortactin
DBN1	Drebrin
DCTN1	Dynactin subunit 1
DIAPH1	Protein diaphanous homolog 1
DLG1	Disks large homolog 1

DLG4	Disks large homolog 4
DNAJB1	DnaJ homolog subfamily B member 2
DNM1	Dynamin-1
DYNC1H1	Cytoplasmic dynein 1 heavy chain 1
EEF1A1	Elongation factor 1-alpha 1
EGFR	Epidermal growth factor receptor
EPB41L3	Band 4.1-like protein 3
EZR	Ezrin
FHL2	Four and a half LIM domains protein 2
FIBP	Acidic fibroblast growth factor intracellular-binding protein
FLNA	Filamin-A
FLNB	Filamin-B
FTL	Ferritin light chain
FYN	Tyrosine-protein kinase Fyn
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCDH	Glutaryl-CoA dehydrogenase, mitochondrial
GFAP	Glial fibrillary acidic protein
GOLGA2	Golgin subfamily A member 2
GRB2	Growth factor receptor-bound protein 2
GSK3A	Glycogen synthase kinase-3 alpha
GSK3B	Glycogen synthase kinase-3 beta
HP	Heterochromatin protein 1
HSCB	Iron-sulfur cluster co-chaperone protein HscB, mitochondrial
HSP90AA1	Heat shock protein HSP 90-alpha
HSP90AB1	Heat shock protein HSP 90-beta
HSPA1A	Heat shock 70 kDa protein 1A
HSPA8	Heat shock cognate 71 kDa protein
HSPB1	Heat shock protein beta-1
IKBKE	Inhibitor of nuclear factor kappa-B kinase subunit epsilon
IMPDH2	Inosine-5'-monophosphate dehydrogenase 2
IRAK2	Interleukin-1 receptor-associated kinase-like 2
JAK3	Tyrosine-protein kinase JAK3
JUN	Transcription factor AP-1
KSR1	Kinase suppressor of Ras 1
LAMTOR1	Ragulator complex protein LAMTOR1
LMNA	Prelamin-A/C
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2
LUC7L2	Putative RNA-binding protein Luc7-like 2
MAD2L1	Mitotic spindle assembly checkpoint protein MAD2A
MAP3K5	Mitogen-activated protein kinase kinase kinase 5
MAPT	Microtubule-associated protein tau
MDM2	E3 ubiquitin-protein ligase Mdm2
MED4	Mediator of RNA polymerase II transcription subunit 4
MEOX2	Homeobox protein MOX-2
MET	Hepatocyte growth factor receptor
MLH1	DNA mismatch repair protein Mlh1
MPP1	55 kDa erythrocyte membrane protein
MYH9	Myosin-9
NIF3L1	NIF3-like protein 1

NUMB	Protein numb homolog
PAK2	Serine/threonine-protein kinase PAK 2
PHB	Prohibitin
PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PLEKHF2	Pleckstrin homology domain-containing family F member 2
PNKD	Probable hydrolase PNKD
PPIA	Peptidyl-prolyl cis-trans isomerase A
PPP1CA	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit
PRKAB1	5'-AMP-activated protein kinase subunit beta-1
PSEN1	Presenilin-1
PTPN1	Tyrosine-protein phosphatase non-receptor type 1
PTPN3	Tyrosine-protein phosphatase non-receptor type 3
PTPN4	Tyrosine-protein phosphatase non-receptor type 4
PTPN5	Tyrosine-protein phosphatase non-receptor type 5
Q53FC7	Heat shock 70kDa protein 6 (HSP70B') variant
Q96BE0	Uncharacterized protein
RCAN1	Calcipressin-1
RET	Proto-oncogene tyrosine-protein kinase receptor Ret
RHEB	GTP-binding protein Rheb
RPL18A	60S ribosomal protein L18a
RPL30	60S ribosomal protein L30
RPP38	Ribonuclease P protein subunit p38
RPS23	40S ribosomal protein S23
RUVBL1	RuvB-like 1
RUVBL2	RuvB-like 2
SCAND1	SCAN domain-containing protein 1
SDF4	45 kDa calcium-binding protein
SHC1	SHC-transforming protein 1
SLC1A2	Excitatory amino acid transporter 2
SLC25A5	ADP/ATP translocase 2
SNCA	Alpha-synuclein
SORT1	Sortilin
SPG21	Maspardin
SPICE1	Spindle and centriole-associated protein 1
SPTAN1	Spectrin alpha chain, non-erythrocytic 1
SRC	Proto-oncogene tyrosine-protein kinase Src
SRI	Sorcin
STAT3	Signal transducer and activator of transcription 3
STIP1	Stress-induced-phosphoprotein 1
STK4	Serine/threonine-protein kinase 4
STUB1	E3 ubiquitin-protein ligase CHIP
TERF1	Telomeric repeat-binding factor 1
TFAP2A	Transcription factor AP-2-alpha
TGOLN2	Trans-Golgi network integral membrane protein 2
TIRAP	Toll/interleukin-1 receptor domain-containing adapter protein
TLE1	Transducin-like enhancer protein 1
TMED2	Transmembrane emp24 domain-containing protein 2
TNPO1	Transportin-1
TP53BP2	Apoptosis-stimulating of p53 protein 2

TTI1	TELO2-interacting protein 1 homolog
TUBB	Tubulin beta chain
UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1
UMPS	Uridine 5'-monophosphate synthase
VCAM1	Vascular cell adhesion protein 1
XIAP	E3 ubiquitin-protein ligase XIAP
YWHAE	14-3-3 protein epsilon
YWHAG	14-3-3 protein gamma
YWHAQ	14-3-3 protein theta
YWHAZ	14-3-3 protein zeta/delta
ZDHHC17	Palmitoyltransferase ZDHHC17

A summary of AD and IS coincident proteins is shown in Figure 6. In Figure 7 are represented all AD proteins retrieved in grey and the coincident proteins also present in the IS pathway are shown in green. Also, the size of the nodes vary according to the amount of control that this node exerts over the interactions of other nodes in the network. In other words, the node will grow according to the number of interactions it performs between communities. This method of analysis allows one to identify proteins with higher impact in different protein pathways.

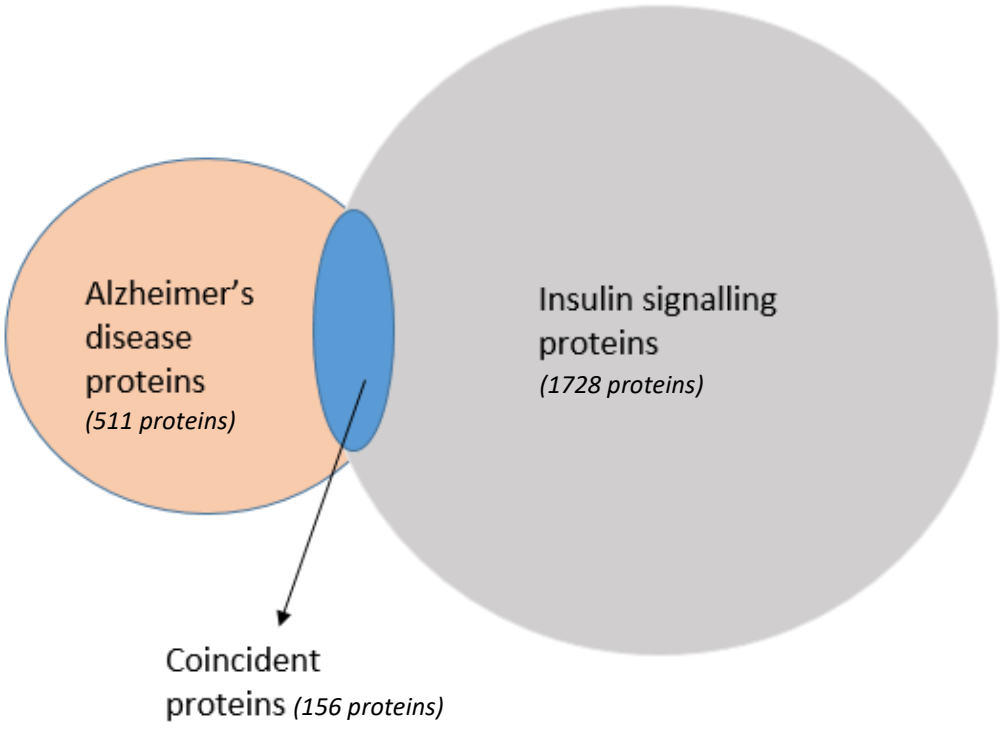

Figure 6- Venn diagram of AD and IS proteins

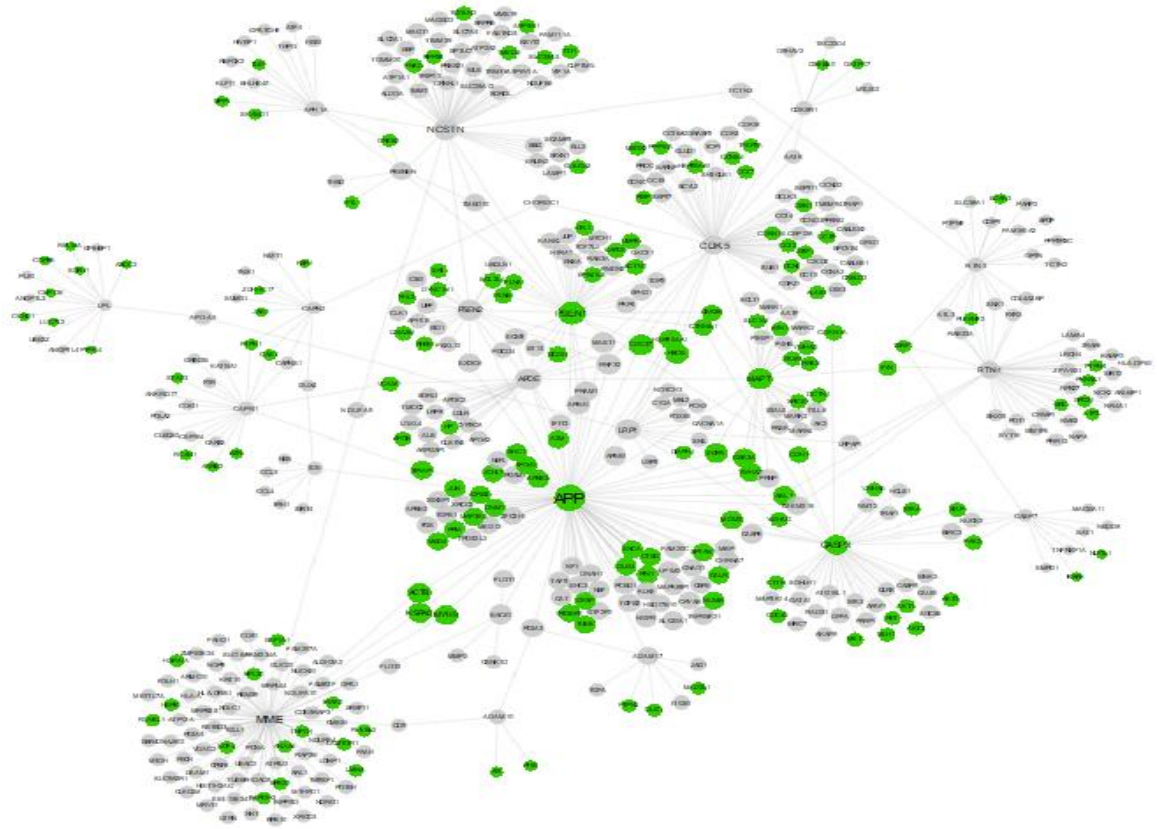

Figure 7- All retrieved AD proteins. Grey nodes correspond to proteins only from AD while green nodes correspond to coincident proteins from AD and IS pathways. Bigger nodes correspond to proteins that are bridges between different communities, re-enforcing their importance by connecting diverse key proteins.

From the Figure 7, APP is clearly a central hub, and can be visualized as the protein with highest impact on AD. On a second plan, a few other proteins appear as central nodes, such as microtubule-associated protein tau (MAPT), presenilin-1 and 2 (PSEN1 and PSEN2), apolipoprotein E (ApoE), caspase-3 (CASP3), cyclin-dependent kinase 5 (CDK5) and Nicastrin (NCSTN).

Tau protein and ApoE also contribute to AD hallmarks, having great importance in the disease development as already discussed. PSEN-1, PSEN-2 and NCSTN are a part of the γ-secretase complex, responsible for APP cleavage. PSEN-1 and 2 mutations can cause autosomal dominant forms of early-onset AD[68], meaning that although they are involved in both pathways of APP cleavage, they play a major role in Aβ formation. Also CASP3 appears prominently and in association with the IS pathway. This protein is involved in the start of the cascade of caspases responsible for execution of apoptosis, leading to neuronal death. Insulin is capable of reducing CASP3 activity in retinal neurons, preventing apoptosis[69]. Impaired insulin signalling may play a pivotal role in the activation of apoptosis cascade. Finally, CDK5 also appears highlighted. A protein from the class of

proline-directed serine/threonine-protein kinases, involved in many important cellular processes such as neuronal cell cycle arrest and differentiation, apoptosis, regulation by phosphorylating key proteins in neuronal survival, axonal and neurite growth, synaptic plasticity and neurotransmission and is also able to phosphorylate Tau[70].

In order to analyse all proteins together, a gene ontology (GO) study was conducted on proteins coincident in the AD and IS pathways. Biological processes and molecular functions were assessed to understand the molecular mechanisms behind AD and also the mechanisms of common proteins between AD and IS pathways. The GO analysis was performed using PANTHER. The output provides the number of proteins involved in the process from the 156 protein input list; a p-value calculated by binomial statistic is also presented (Tables 12 and 13). This p-value represents the probability that the number of genes observed in the category occurred by chance, in comparison with a reference database list. A small p-value indicates that the observed number is significant and potentially interesting.

Regarding the 511 proteins in the AD interactome network from this study, and with respect to biological processes (Table 8), the response to stimulus appeared with the highest number of hits, indicating that many of the proteins are key signal transducers. However, by considering the p-value, other processes appear to be more significant. With the lowest p-value being obtained for cellular component organization, which in AD is completely deregulated. From this analysis, more than 50% of the AD set of proteins appears to be involved in this process. In this set, all microtubule and microtubule associated proteins appear, namely microtubule-associated protein tau, one of the major proteins involved in AD. Furthermore, many nodes also appear to be associated with cell communication and signalling, with almost 50% of our dataset of proteins present in these categories and with relatively low p-values. This is suggestive of a high impact that this disease can have in cell signalling.

In the molecular functions (Table 9), with 90% of representation and an absurdly low p-value, we identified protein binding. Suggesting that this disease is not dependent on proteins alone but more protein complexes, which are more difficult to target than a single protein. It also reinforces that AD is a complex disease and many proteins can influence it. Of note, receptor binding was one of the top hits, with 20% of proteins from the set being able to bind to a receptor, probably leading to cascades of events. These cascades will lead to phosphorylation of other proteins, involving protein kinases and protein kinase binding, which was a molecular function associated with 14% of proteins (Table 9). This is a highly relevant molecular function, for instance playing an important role in tau hyperphosphorylation.

Table 10- Biological process analysis of interesting processes from AD proteins.

Biological process	Number of proteins	% from total	P value
Response to stimulus	339/511	66%	6.03E-12
Cellular component organization	282/511	55%	3.59E-40
Cell communication	244/511	48%	8.39E-21
Signalling	238/511	47%	1.74E-19

Table 11- Molecular function analysis of interesting functions from AD proteins.

Molecular function	Number of proteins	% from total	P value
Protein binding	461/511	90%	1.36E-57
Receptor binding	103/511	20%	9.34E-16
Kinase binding	73/511	14%	1.38E-22

For only the coincident proteins, the same analyses with the same parameters were performed. Interestingly, all biological processes and molecular functions (Table 10 and 11) have an increase in the percentage of representation. All the identified processes have an important impact on AD and the fact that all increased can mean that insulin signalling probably plays a pivotal role in many key proteins associated with AD.

Table 12- Biological process analysis of interesting processes from AD core proteins and only coincident proteins with IS pathway.

Biological process	Number of proteins	% from total	P value
Response to stimulus	120/156	77%	1.51E-18
Cellular component organization	104/156	67%	3.79E-23
Cell communication	90/156	58%	5.07E-13
Signalling	88/156	56%	2.44E-12

Table 13- Molecular function analysis of interesting functions from AD core proteins and only coincident proteins with IS pathway.

Molecular function	Number of proteins	% from total	P value
Protein binding	152/156	97%	5.26E-30
Receptor binding	46/156	30%	1.46E-12
Kinase binding	42/156	27%	1.71E-23

4.3. SH-SY5Y differentiation

Having established a strong correlation at a systems biology level, namely a strong overlap in the AD and IS networks it was deemed relevant to pursue correlations using a neuronal cell model.

The literature offers many methods to differentiate the SH-SY5Y cell line such as to achieve a neuronal like morphology. Upon consulting various protocols, the concentration of medium FBS and the exposure time of RA were the variants less defined. Thus, in this study, contrast-phase photographs were taken at day 0, day 3 and day 5 to visualize morphological differences in undifferentiated and differentiated cells in the presence of 1% and 10% FBS supplemented with 10 µM of RA.

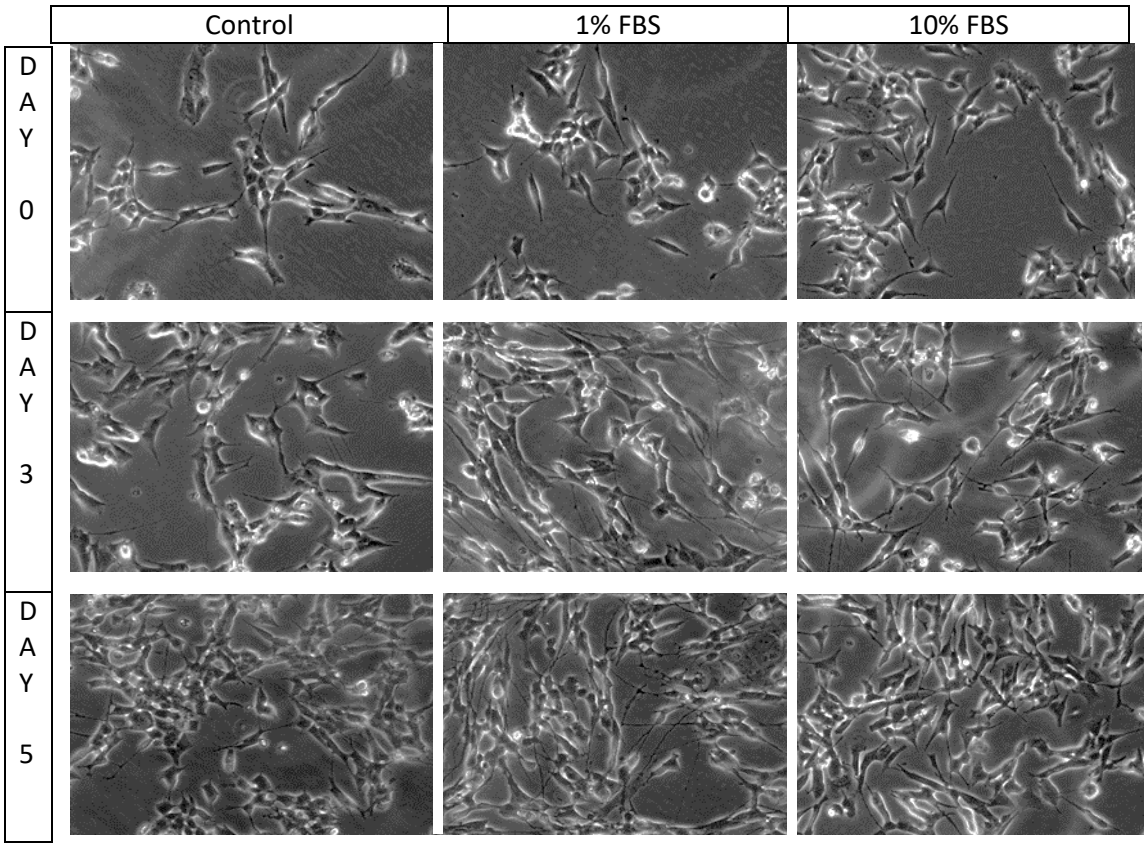

Figure 8- Differentiation of SH-SY5Y cell line at different time points. The control is undifferentiated cells with complete growth medium. Both 1% FBS and 10% FBS were supplemented with 10 µM of RA.

Day 0 was the day after the seeding and no treatment had yet been performed, so all cells looked the same. At day 3, one can already distinguish 1% FBS and 10% FBS from the undifferentiated control. A more elongated phenotype and more neurites can be visualized in the 1% and 10% FBS concentrations, but no major differences between them. At day 5, the cells in 1% FBS appear to have formed much more neurites than the cells in 10% FBS or the control. The 5 day at 1% FBS protocol induced cells into a more mature neuron type and for this reason, this was the protocol used for the rest of the experiments.

4.4. Western blot analysis

Western blots were conducted using SH-SY5Y differentiated cells for 5 days with 10 µM of RA and 1% FBS medium and treated with 0, 1, 10 and 100 nM of human insulin for 0, 10 and 60 minutes to assess the effect of insulin in AD related proteins. For this purpose, the levels of total tau, tau phosphorylated at serine 396, total APP and APP phosphorylated at threonine 668 were assessed.

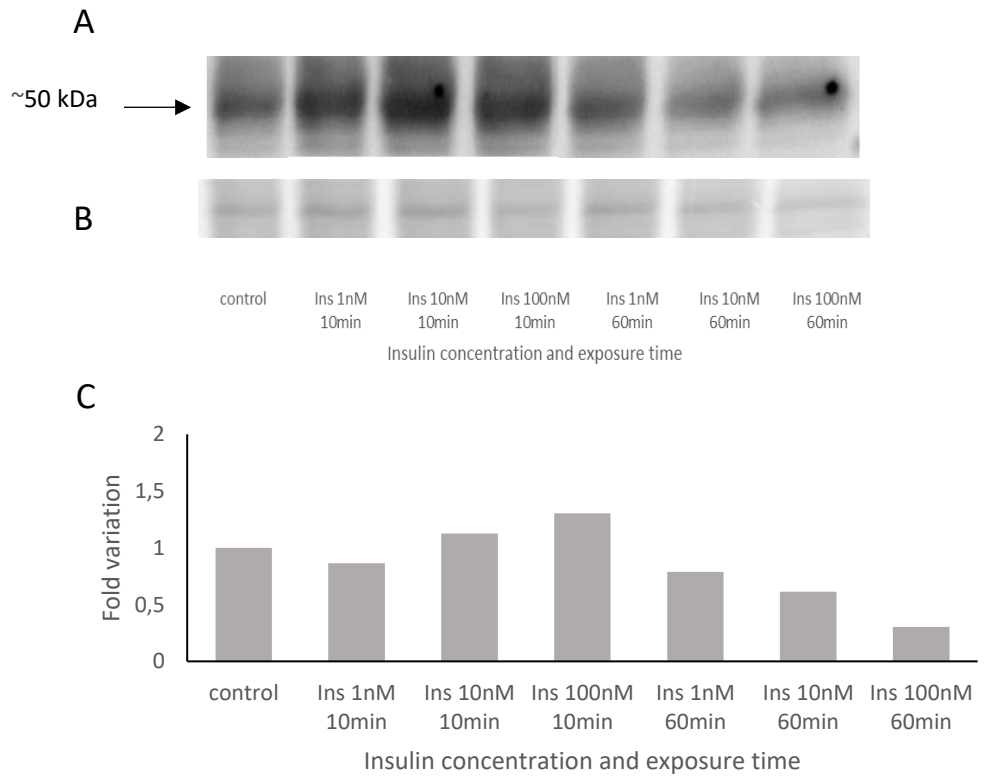

Figure 9- Total Tau expression in SH-SY5Y differentiated cells treated with insulin. A- Western blot analysis of SH-SY5Y lysates treated with different concentrations and exposure times of insulin. B- Ponceau staining as loading control. C- Comparison of total Tau expression levels in different concentration and exposure times of insulin.

Regarding the levels of total Tau protein, a very small increase can be detected in the 10 minutes exposure to insulin, mainly in the 100nM (higher concentration), followed by a decrease after 60 minutes exposure. This decrease may be explained by cellular death due to toxic concentrations of insulin. It is possible to deduce that insulin does not appear to influence the expression of total Tau protein, at least during small exposure periods, as tested here.

For the phosphorylation of protein Tau, many residues appear phosphorylated in AD. The choice to study the serine 396 come from previous work from our laboratory pointing to a significant increase of phosphorylation at this residue in SH-SY5Y cells exposed to the Aβ peptide[23] and its importance in the formation of paired helical Tau filaments (PHF).

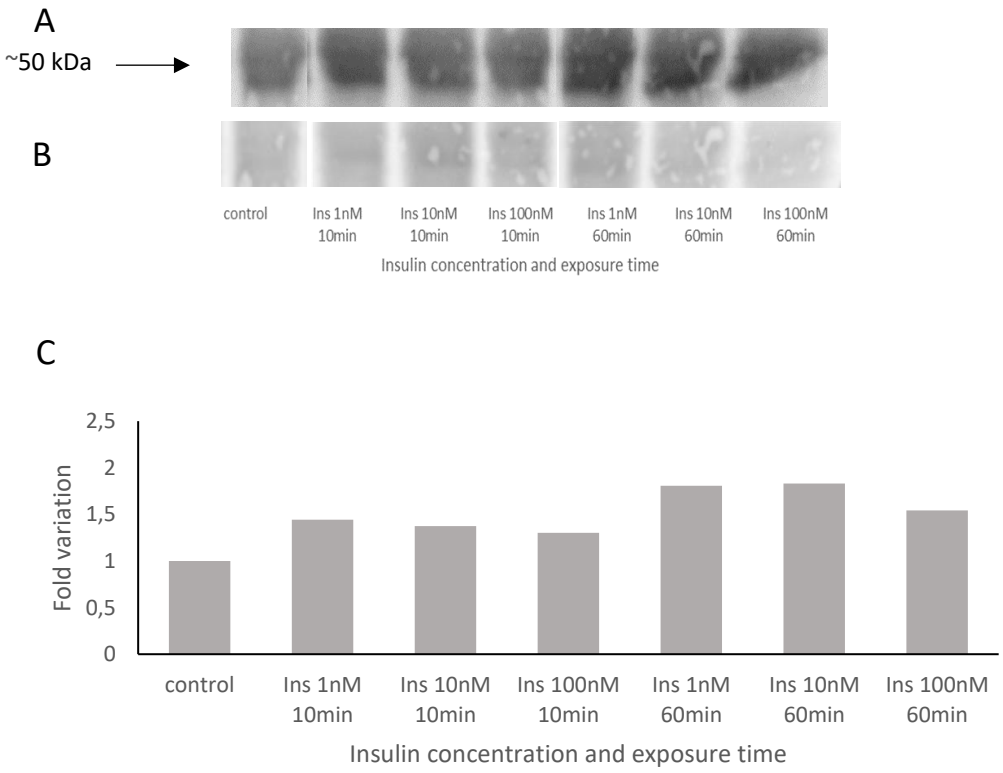

Figure 10- Tau phosphorylation at serine 396 in SH-SY5Y differentiated cells treated with insulin. A- Western blot analysis of SH-SY5Y lysates treated with different concentrations and exposure times of insulin. B- Ponceau straining as loading control. C- Comparison of Tau phosphorylation at serine 396.

In relation to the phosphorylation of protein Tau at the serine 396, an increase can be detected independent of the concentrations of insulin and more dependent on the exposure time.

In order to better visualize the data referent to total Tau and Tau phosphorylated at serine 396, a ratio was performed (Figure 11).

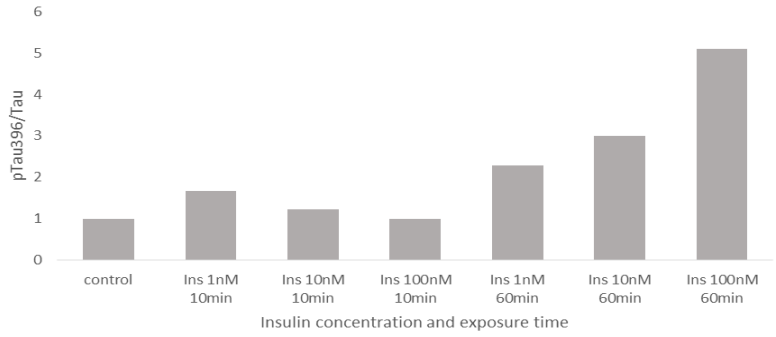

Figure 11- Ratio between Tau phosphorylated at the serine 396 and total Tau.

From the ratio, it is possible to see that higher exposure times and higher concentrations of insulin can lead to an effect on Tau protein, but further studies are necessary to enable any conclusion.

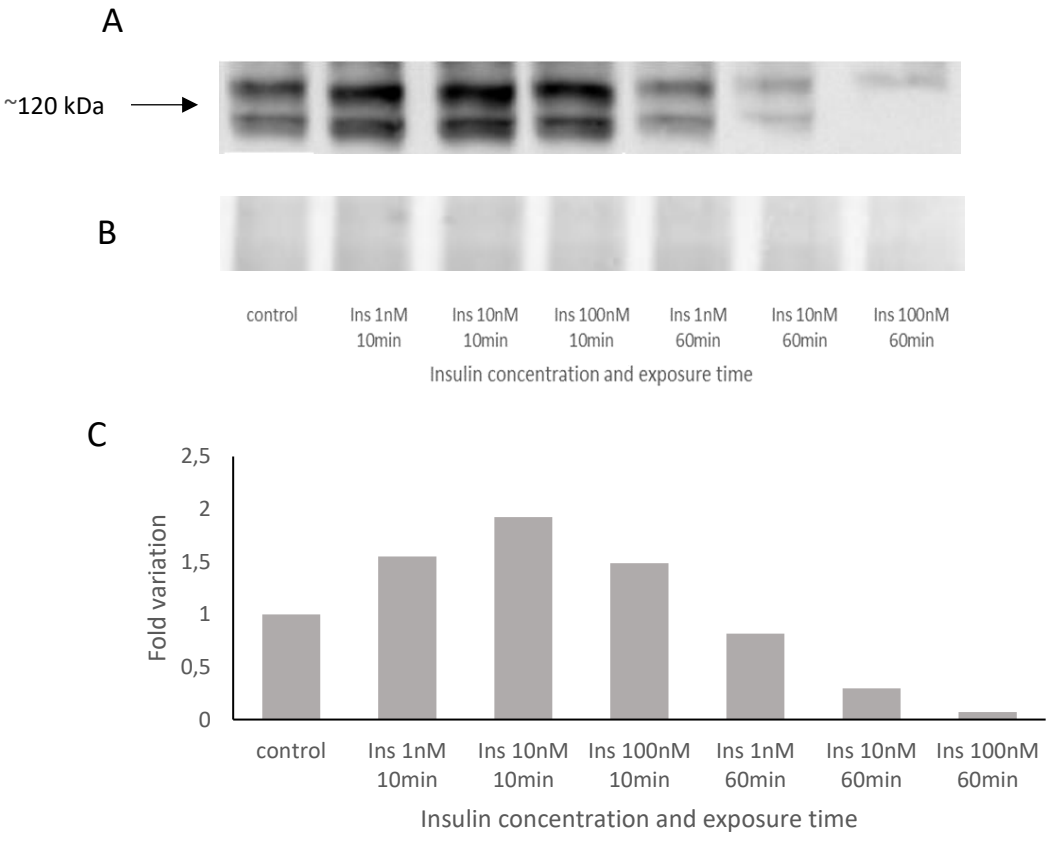

Figure 12- Total APP expression in SH-SY5Y differentiated cells treated with insulin. A- Western blot analysis of SH-SY5Y lysates treated with different concentrations and exposure times of insulin. B- Ponceau straining as loading control. C- Comparison of total APP expression levels in different concentrations and exposure times of insulin.

Regarding APP, in the 10 minutes of exposure to insulin, an increase in the expression can be observed. However, while the SH-SY5Y cells were exposed to insulin for 60 minutes, a drastic decreased of total levels of APP in the higher concentrations can be noticed. A study performed in mice showed that the brain insulin receptor signalling mediated APP processing[71], that is concurrent with the 60 minutes results reported. From the data here presented it seems reasonable to deduce that even short exposures to insulin appear to increase APP expression and possible APP processing. However to proof this further experimentation is necessary.

APP processing is intrinsically associated with its phosphorylation state. This applies to its intracellular trafficking as well as cleavage via different pathways and even Aβ production[72,73]. Thus the phosphorylation at threonine 668 of APP was assessed. The phosphorylation of this specific residue was previously shown to be involved APP cleavage, promoting the amyloidogenic pathway[10].

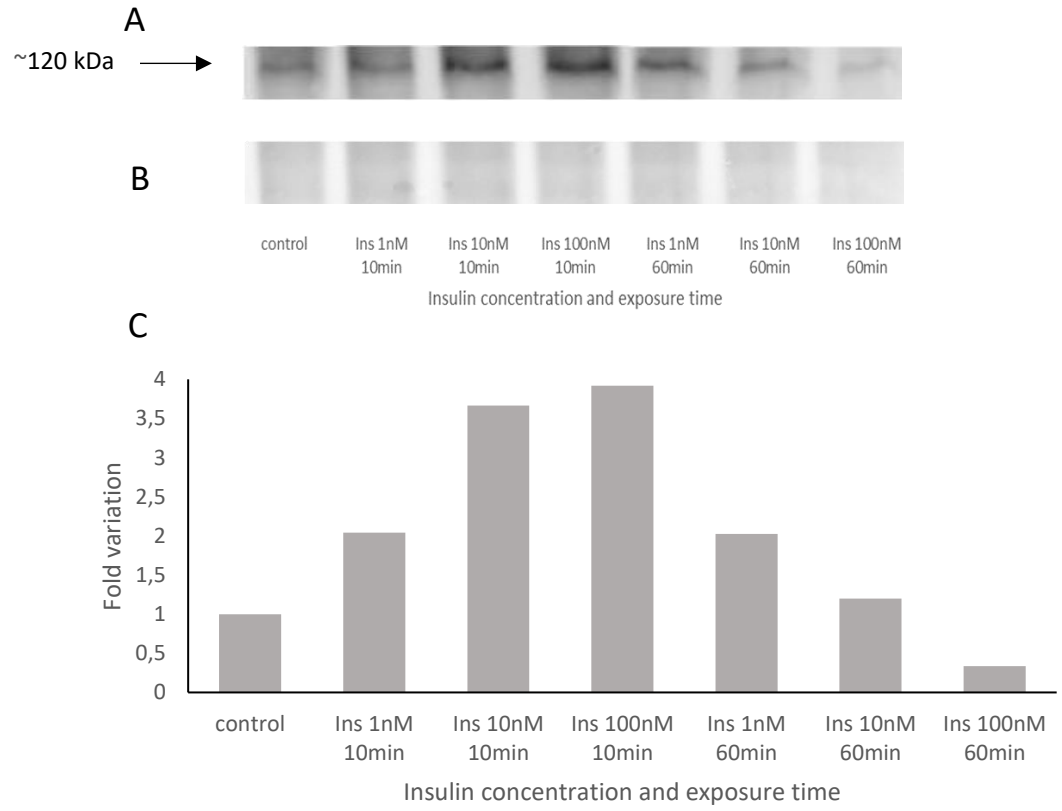

Figure 13- APP phosphorylation at threonine 668 in SH-SY5Y differentiated cells treated with insulin. A- Western blot analysis of SH-SY5Y lysates treated with different concentrations and exposure times of insulin. B- Ponceau straining as loading control. C- Comparison of APP phosphorylation at threonine 668 in different concentrations and exposure times of insulin.

It is possible to visualize a high increase in the phosphorylation of APP thr668 when exposed for 10 minutes to different concentration of insulin, followed by a decrease at 60 minutes of exposure. From these results it is possible to conclude that the insulin signalling may actually play a role in determining APP phosphorylation and be putatively involved in Aβ production. The decrease in the phosphorylation of this residue at 60 minutes of insulin exposure may be related to a decrease in total APP. This question deserves to be further investigated.

Regarding the literature, almost all research conducted in insulin and AD report an anti-amyloidogenic effect of insulin, due to a decrease in phosphorylation of APP at threonine 668, inactivation of GSK3β and decrease of β-secretase activity[74]. A study conducted in SH-SY5Y cells relate the decreasing phosphorylation of APP thr668 with the same concentrations of insulin used in this study, but for a higher exposure time (24h)[74] and this appeared to be beneficial to AD. Another study made the same assumptions based in reduced APP Thr668 phosphorylation in long term administration in young adult mice with AD phenotype with intranasal administration of insulin[75]. While the data here presented also reveals a decreased phosphorylation at 60 minutes the shorter exposure period is contradictory. Namely shorter exposure periods appear to result in increased phosphorylation of APP-Thr668.

Insulin may actually play an important role as an anti-amyloidogenic but the results obtained in this study show that it may not be as linear as initially thought. The level of APP phosphorylation on Thr668 appears to be concentration and time of exposure dependent. Clearly the issue has to be further investigated.

4.5. Resazurin assay analysis

A resazurin assay was performed to assess cell viability to confirm that the obtained results were real and not due to insulin toxicity and cellular death.

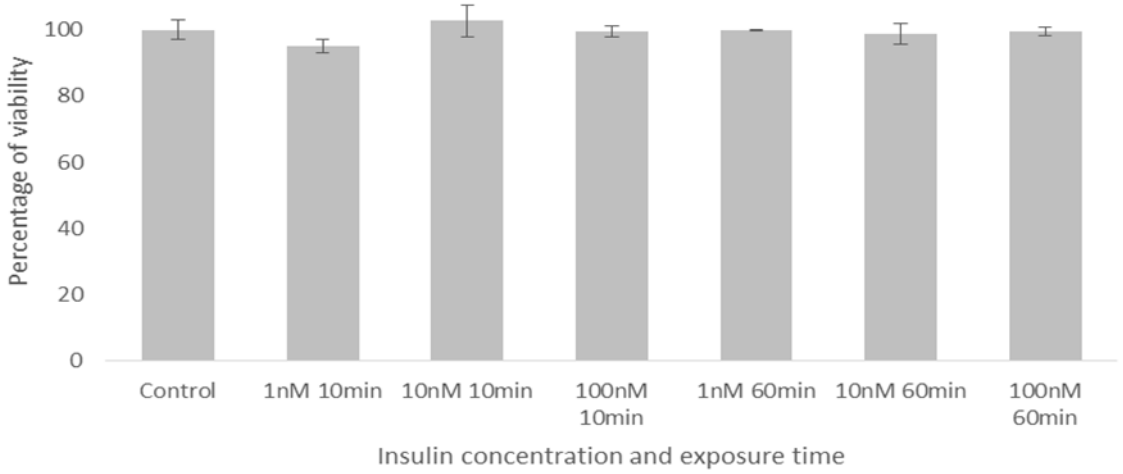

Figure 14 -Resazurin assay. Viability of SH-SY5Y differentiated cells pre-treated with 0, 1, 10 and 100 nM of insulin for 0, 10 and 60 minutes.

As it can be seen in the Figure 13, no differences can be observed from cell viability, discarding the hypothesis that insulin could toxically interfere with the results. On the other hand, insulin can still be toxic at higher exposure times, as it can lead to Aβ production. This however, was not tested.

5. Conclusion

5. Conclusion

Growing evidence links impaired brain insulin signalling and insulin resistance to the development of AD. A correlation between diabetes and poor cognitive scores in MMSE tests were observed, linking diabetes to a deteriorating cognition. Additionally, carriers of the allele ApoE-ε2 showed to be protected against diabetes, as seen for AD.

Nonetheless, the majority of proteins in AD and in IS pathways, were shown to be present in the synapse, re-enforcing their roles in synaptic signalling and plasticity. Coincident proteins on AD and IS pathways had 96% of representation in the synapse proteome, strengthening their role in the synapse as well as the relevance of IS to AD.

A gene ontology study was also conducted, in order to assess the most important biological processes and molecular function of the proteins involved. As for AD, response to stimulus, cellular component organization, cell communication, signalling, protein binding, receptor binding and kinase binding were categories with elevated representation. Regarding coincident proteins between AD and IS pathways, an increase in all categories was observed, suggesting that insulin plays a pivotal role in many AD related events.

Finally, the analysis of western blots performed in differentiated SH-SY5Y cells treated with different concentrations and exposure times of insulin displayed interesting preliminary results. For Tau protein, a decrease in the intracellular levels of total Tau and an increase in the phosphorylation of serine 396 was observed, with clear evidence when a ration between Tau phosphorylated and total Tau is performed. On the other hand, regarding APP, an increase in the expression at 10 minutes of exposure followed by a decrease at 60 minutes of insulin exposure was observed. This can mean that insulin plays a role in the expression/processing of APP. As for the phosphorylation of threonine 668 of APP, which, in the literature, has been shown to favour Aβ production by promoting the cleavage by amyloidogenic pathway, was increased in the cells treated with insulin at 10 minutes, leading one to propose that short exposure to insulin may actually promote Aβ production.

Insulin signalling plays an unquestionable role in memory and cognition, and consequently in AD, but further work is necessary to understand all the mechanisms behind these cascades of events.

6. References

6. References

1. Martin Prince, A. *et al.* World Alzheimer Report 2015 The Global Impact of Dementia Analysis of prevalence, incidence, cost and trends. (2015).

2. Querfurth, H. W. & LaFerla, F. M. Alzheimer's Disease. *N. Engl. J. Med.* **362,** 329–344 (2010).

3. Szekely, C. A. *et al.* Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. *Neuroepidemiology* **23,** 159–69 (2004).

4. Waxman, S. G. *From neuroscience to neurology : neuroscience, molecular medicine, and the therapeutic transformation of neurology*. (Elsevier Academic Press, 2005).

5. Martins, I. J. *et al.* Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. *Mol. Psychiatry* **11,** 721–736 (2006).

6. Salawu, F., Umar, J. & Olokoba, A. Alzheimer's disease: A review of recent developments. *Ann. Afr. Med.* **10,** 73 (2011).

7. Schmand, B., Smit, J. H., Geerlings, M. I. & Lindeboom, J. The effects of intelligence and education on the development of dementia. A test of the brain reserve hypothesis. *Psychol. Med.* **27,** 1337–44 (1997).

8. Fleminger, S., Oliver, D. L., Lovestone, S., Rabe-Hesketh, S. & Giora, A. Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *J. Neurol. Neurosurg. Psychiatry* **74,** 857–62 (2003).

9. Mittal, K., Mani, R. J. & Katare, D. P. Type 3 Diabetes: Cross Talk between Differentially Regulated Proteins of Type 2 Diabetes Mellitus and Alzheimer's Disease. *Sci. Rep.* **6,** 25589 (2016).

10. Lee, M.-S. *et al.* APP processing is regulated by cytoplasmic phosphorylation. *J. Cell Biol.* **163,** 83–95 (2003).

11. Kamenetz, F. *et al.* APP Processing and Synaptic Function. *Neuron* **37,** 925–937 (2003).

12. Wang, J.-Z., Xia, Y.-Y., Grundke-Iqbal, I. & Iqbal, K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J. Alzheimers. Dis.* **33 Suppl 1,** S123-39 (2013).

13. Oliveira, J., Costa, M., De Almeida, M. S. C., Da Cruz E Silva, O. A. B. & Henriques, A. G. Protein Phosphorylation is a Key Mechanism in Alzheimer's Disease. *J. Alzheimer's Dis.* **58,** 953–978 (2017).

14. Huang, W.-J., Zhang, X. & Chen, W.-W. Role of oxidative stress in Alzheimer's disease. *Biomed. reports* **4,** 519–522 (2016).

15. Ring, S. *et al.* The Secreted -Amyloid Precursor Protein Ectodomain APPs Is Sufficient to Rescue the Anatomical, Behavioral, and Electrophysiological Abnormalities of APP-Deficient Mice. *J. Neurosci.* **27,** 7817–7826 (2007).

16. O'Brien, R. J. & Wong, P. C. Amyloid precursor protein processing and Alzheimer's disease. *Annu. Rev. Neurosci.* **34,** 185–204 (2011).

17. Kojro, E. & Fahrenholz, F. The non-amyloidogenic pathway: structure and function of alpha-secretases. *Subcell. Biochem.* **38,** 105–27 (2005).

18. Thinakaran, G. & Koo, E. H. Amyloid Precursor Protein Trafficking, Processing, and Function. *J. Biol. Chem.* **283,** 29615–29619 (2008).

19. Pająk, B., Kania, E. & Orzechowski, A. Killing Me Softly: Connotations to Unfolded Protein Response and Oxidative Stress in Alzheimer's Disease. *Oxid. Med. Cell. Longev.* 1–17 (2016).

20. Wischik, C. M., Crowther, R. A., Stewart, M. & Roth, M. Subunit structure of paired helical filaments in Alzheimer's disease. *J. Cell Biol.* **100,** 1905–12 (1985).

21. Qiang, L., Yu, W., Andreadis, A., Luo, M. & Baas, P. W. Tau Protects Microtubules in the Axon from Severing by Katanin. *J. Neurosci.* **26,** 3120–3129 (2006).

22. Šimić, G. *et al.* Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. *Biomolecules* 6 (2016).

23. Oliveira, J. M., Henriques, A. G., Martins, F., Rebelo, S. & Da Cruz E Silva, O. A. B. Amyloid-β Modulates Both AβPP and Tau Phosphorylation. *J. Alzheimer's Dis.* **45,** 495–507 (2015).

24. Boucher, J., Kleinridders, A. & Kahn, C. R. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb. Perspect. Biol.* **6,** (2014).

25. Chang, L., Chiang, S.-H. & Saltiel, A. R. Insulin signaling and the regulation of glucose transport. *Mol. Med.* **10,** 65–71 (2004).

26. Van Obberghen, E. *et al.* Surfing the insulin signaling web. *Eur. J. Clin. Invest.* **31,** 966–977 (2001).

27. Lizcano, J. M. & Alessi, D. R. The insulin signalling pathway. *Curr. Biol.* **12,** R236-8 (2002).

28. Dong Chen, D., Waters, S. B., Holt, K. H. & Pessin, J. E. SOS phosphorylation and disassociation of the Grb2-SOS complex by the ERK and JNK signaling pathways. *J. Biol. Chem.* **271,** 6328–32 (1996).

29. Gray, S. M., Meijer, R. I. & Barrett, E. J. Insulin regulates brain function, but how does it get there? *Diabetes* **63,** 3992–7 (2014).

30. Blázquez, E., Velázquez, E., Hurtado-Carneiro, V. & Ruiz-Albusac, J. M. Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front. Endocrinol.* **5,** 161 (2014).

31. Plum, L., Schubert, M. & Brüning, J. C. The role of insulin receptor signaling in the brain. *Trends Endocrinol. Metab.* **16,** 59–65 (2005).

32. Hui, H., Tang, G. & Go, V. Hypoglycemic herbs and their action mechanisms. *Chin. Med.* **4,** 11 (2009).

33. Kashyap, S. *et al.* A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes* **52,** 2461–74 (2003).

34. Kahn, S. E., Hull, R. L. & Utzschneider, K. M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* **444,** 840–846 (2006).

35. Group, D. P. P. R. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. *N. Engl. J. Med.* **346,** 393–403 (2002).

36. Gutiérrez-Rodelo, C., Roura-Guiberna, A. & Olivares-Reyes, J. A. Molecular Mechanisms of Insulin Resistance: An Update.

37. Hoyer, S. Der Aminosauren-Stoffwechsel des normalen menschlichen Gehirns. *Klin. Wochenschr.* **48,** 1239–1243 (1970).

38. Talbot, K. *et al.* Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* **122,** 1316–38 (2012).

39. Duelli, R., Schröck, H., Kuschinsky, W. & Hoyer, S. Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. *Int. J. Dev. Neurosci.* **12,** 737–43 (1994).

40. Wang, X. *et al.* Insulin inhibits Aβ production through modulation of APP processing in a cellular model of Alzheimer's disease. *Neuroendocr. Lett Neuroendocrinol. Lett.* **35,** 224–229 (2014).

41. Welsh, G. I. & Proud, C. G. Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochem. J.* **294 (Pt 3),** 625–9 (1993).

42. Troussard, A. A., Tan, C., Yoganathan, T. N. & Dedhar, S. Cell-extracellular matrix interactions stimulate the AP-1 transcription factor in an integrin-linked kinase- and glycogen synthase kinase 3-dependent manner. *Mol. Cell. Biol.* **19,** 7420–7 (1999).

43. Turenne, G. A. & Price, B. D. Glycogen synthase kinase3 beta phosphorylates serine 33 of p53 and activates p53's transcriptional activity. *BMC Cell Biol.* **2,** 12 (2001).

44. Anderton, B. H. *et al.* Sites of phosphorylation in tau and factors affecting their regulation. *Biochem. Soc. Symp.* 73–80 (2001).

45. Brion, J. P. *et al.* Neurofibrillary tangles and tau phosphorylation. *Biochem. Soc. Symp.* 81–8 (2001).

46. Hernández, F., Langa, E., Cuadros, R., Avila, J. & Villanueva, N. Regulation of GSK3 isoforms by phosphatases PP1 and PP2A. *Mol. Cell. Biochem.* **344,** 211–215 (2010).

47. Vintém, A. P. B., Henriques, A. G., da Cruz e Silva, O. A. B. & da Cruz e Silva, E. F. PP1 inhibition by Aβ peptide as a potential pathological mechanism in Alzheimer's disease. *Neurotoxicol. Teratol.* **31,** 85–88 (2009).

48. Yu, J.-T., Tan, L. & Hardy, J. Apolipoprotein E in Alzheimer's Disease: An Update. *Annu. Rev. Neurosci.* **37,** 79–100 (2014).

49. Mahley, R. W., Weisgraber, K. H. & Huang, Y. Apolipoprotein E4: A causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc. Natl. Acad. Sci.* **103,** 5644–5651 (2006).

50. Filippi, B. M., Mighiu, P. I. & Lam, T. K. T. Is Insulin Action in the Brain Clinically Relevant?

Diabetes **61,** 773–775 (2012).

51. Reger, M. A. *et al.* Effects of intranasal insulin on cognition in memory-impaired older adults: Modulation by APOE genotype. *Neurobiol. Aging* **27,** 451–458 (2006).

52. Reger, M. A. *et al.* Intranasal insulin improves cognition and modulates -amyloid in early AD. *Neurology* **70,** 440–448 (2008).

53. Craft, S. *et al.* Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* **28,** 809–22 (2003).

54. Chan, E. S., Chen, C., Cole, G. M. & Wong, B.-S. Differential interaction of Apolipoprotein-E isoforms with insulin receptors modulates brain insulin signaling in mutant human amyloid precursor protein transgenic mice. *Sci. Rep.* **5,** 13842 (2015).

55. Qiu, W. Q. *et al.* Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J. Biol. Chem.* **273,** 32730–8 (1998).

56. Vekrellis, K. *et al.* Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci.* **20,** 1657–65 (2000).

57. Lee, Y. H. & White, M. F. Insulin receptor substrate proteins and diabetes. *Arch. Pharm. Res.* **27,** 361–70 (2004).

58. Zhang, M., Lv, X.-Y., Li, J., Xu, Z.-G. & Chen, L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp. Diabetes Res.* **2008,** 704045 (2008).

59. Gupta, A., Bisht, B. & Dey, C. S. Peripheral insulin-sensitizer drug metformin ameliorates neuronal insulin resistance and Alzheimer's-like changes. *Neuropharmacology* **60,** 910–920 (2011).

60. Rosa, I. M., Henriques, A. G., Carvalho, L., Oliveira, J. & da Cruz e Silva, O. A. B. Screening Younger Individuals in a Primary Care Setting Flags Putative Dementia Cases and Correlates Gastrointestinal Diseases with Poor Cognitive Performance. *Dementia and Geriatric Cognitive Disorders.* **43,** 15–28 (2017).

61. Morris, J. C. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* **43,** 2412–4 (1993).

62. Yesavage, J. A. & Sheikh, J. I. 9/Geriatric Depression Scale (GDS). *Clin. Gerontol.* **5,** 165–173 (1986).

63. Katz, S. Assessing self-maintenance: activities of daily living, mobility, and instrumental activities of daily living. *J. Am. Geriatr. Soc.* **31,** 721–7 (1983).

64. Lawton, M. P. & Brody, E. M. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* **9,** 179–86 (1969).

65. Morgado, J., Rocha, C., Maruta, C., Guerreiro, M. & Martins, I. Morgado: Novos valores normativos do mini-mental.

66. Morgado, J., Rocha, C. S., Maruta, C., Guerreiro, M. & Martins, I. P. Cut-off scores in MMSE: a moving target? *Eur. J. Neurol.* **17,** 692–695 (2010).

67. Rosa, I., Henriques, A. & Da Cruz e Silva, O. Cognitive Performance of Primary Care based Cohort using Mini Mental State Examination.

68. Lanoiselée, H.-M. *et al.* APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med.* **14,** e1002270 (2017).

69. Barber, A. J. *et al.* Insulin rescues retinal neurons from apoptosis by a phosphatidylinositol 3-kinase/Akt-mediated mechanism that reduces the activation of caspase-3. *J. Biol. Chem.* **276,** 32814–21 (2001).

70. Kimura, T., Ishiguro, K. & Hisanaga, S.-I. Physiological and pathological phosphorylation of tau by Cdk5. *Front. Mol. Neurosci.* **7,** 65 (2014).

71. Stöhr, O. *et al.* Insulin receptor signaling mediates APP processing and β-amyloid accumulation without altering survival in a transgenic mouse model of Alzheimer's disease. *Age (Dordr).* **35,** 83–101 (2013).

72. Gandy, S. *et al.* Amyloid Precursor Protein Sorting and Processing: Transmitters, Hormones, and Protein Phosphorylation Mechanisms. *Intracellular Traffic and Neurodegenerative Disorders* 1–9.

73. Henriques, A. G. *et al.* Altered protein phosphorylation as a resource for potential AD biomarkers. *Sci. Rep.* **6,** 30319 (2016).

74. Pandini, G. *et al.* Insulin Has Multiple Antiamyloidogenic Effects on Human Neuronal Cells. *Endocrinology* **154,** 375–387 (2013).

75. Mao, Y.-F. *et al.* Intranasal insulin alleviates cognitive deficits and amyloid pathology in young adult APPswe/PS1dE9 mice. *Aging Cell* **15,** 893–902 (2016).

7. Appendix

7. Appendix

Cell Culture Solutions

- **PBS (1x)**

For a final volume of 500 mL, dissolve one pack of BupH Modified Dulbecco's Phosphate Buffered Saline Pack (Pierce) in deionized H_2O. Final composition:

- 8 mM Sodium Phosphate
- 2 mM Potassium Phosphate
- 140 mM Sodium Chloride
- 10 mM Potassium Chloride

Sterilize by filtering through a 0.2 µm filter and store at 4°C.

- **10% FBS MEM:F12 (1:1)**
  - 4.805 g MEM
  - 5.315 g F12
  - 1.5 g NaHCO3
  - 0.055 g Sodium Pyruvate
  - 10 mL Streptomycin/Penicillin/Amphotericin solution
  - 100 mL 10% FBS
  - 2.5 mL L-glutamine (200 mM stock solution)

Dissolve in deionized H_2O. Adjust the pH to 7.2-7.3. Adjust the volume to 1000 mL with deionized H_2O.
For other combinations of FBS, replace 100 mL FBS with 30 mL (3% FBS MEM:F12), 10 mL (1% FBS MEM:F12) or remove FBS (serum-free MEM:F12).

Western Blot Solutions

- **LGB (lower gel buffer) (4x)**

To 900 mL of deionized H_2O add:

- 181.65 g of Tris
- 4 g of SDS

Mix until the solutes have dissolved. Adjust pH to 8.9 and adjust the volume to 1 L with deionized H_2O.

- **UGB (Upper gel buffer) (4x)**

To 900 mL of deionized H_2O add:

- 75.69 g of Tris

Mix until the solute has dissolved. Adjust the pH to 6.8 and adjust the volume to 1 L with deionized H_2O.

- **10% APS (ammonium persulfate)**

In 10 mL of deionized H_2O dissolve 1 g of APS. Note: prepare fresh before use.

- **10% SDS (sodium dodecilsulfate)**

In 10 mL of deionized H_2O dissolve 1 g of SDS.

- **Loading Gel Buffer (4x)**
  - 2.5 mL 1M Tris solution (pH 6.8) 2.5 mL (250 mM)
  - 0.8 g SDS (8%)
  - 4 mL Glicerol (40%)
  - 2 mL β-mercaptoethanol (2%)
  - 1 mg Bromofenol blue (0.01%)

Adjust the volume to 10 mL with deionized H_2O. Store in darkness at room temperature.

- **1 M Tris (pH 6.8) solution**

To 150 mL of deionized H_2O add:
  - 30.3 g Tris base

Adjust the pH to 6.8 and adjust the final volume to 250 mL with deionized H_2O.

- **10x Running Buffer**
  - 30.3 g Tris (250 mM)
  - 144.2 g Glycine (2.5 M)
  - 10 g SDS (1%)

  Dissolve in deionized H_2O, adjust the pH to 8.3 and adjust the volume to 1 L.

Supplementary table 1- All synapse proteins

A0A024R	ACHE	ADGRA2	AIFM3	AMFR	ANXA2	APPL1	ARHGEF1
5S0	ACIN1	ADGRA3	AIG1	AMIGO2	ANXA6	APTX	9
A0A1B0G	ACLY	ADGRB2	AIM1	AMMECR	ANXA7	AQP1	ARHGEF2
TW0	ACOT7	ADGRB3	AIM2	1	AOAH	AQP3	ARHGEF3
A1BG	ACP1	ADGRL1	AIMP2	AMOT	AOC3	AR	ARHGEF4
A1DRY3	ACP6	ADGRL2	AIP	AMOTL1	AOPEP	ARAP2	ARHGEF5
A2M	ACSBG1	ADH6	AIPL1	AMOTL2	AP1B1	ARAP3	ARHGEF6
A4GALT	ACSL3	ADIRF	AIRE	AMPD3	AP1G1	ARC	ARHGEF7
A6NNT2	ACSL4	ADNP	AJAP1	AMPH	AP1G2	ARF1	ARID3A
A8K1F4	ACSL5	ADO	AK2	AMZ2	AP1M1	ARF4	ARID4B
A8KAD6	ACTA1	ADORA1	AK3	ANAPC10	AP1M2	ARF5	ARID5A
AAK1	ACTA2	ADORA2	AK5	ANAPC15	AP1S1	ARF6	ARID5B
AAMDC	ACTB	A	AK8	ANAPC4	AP1S2	ARFGAP1	ARIH2
AARD	ACTBL2	ADRA1B	AKAP1	ANAPC5	AP1S3	ARFGAP3	ARKL1
AARSD1	ACTC1	ADRA1D	AKAP10	ANAPC7	AP2A1	ARFGEF1	ARL1
AASS	ACTG1	ADRA2B	AKAP11	ANG	AP2A2	ARFGEF2	ARL15
AATK	ACTG2	ADRA2C	AKAP12	ANGEL2	AP2B1	ARFGEF3	ARL16
ABCA1	ACTL6B	ADRB1	AKAP14	ANGPT1	AP2M1	ARFIP1	ARL2BP
ABCA2	ACTL7A	ADRB2	AKAP17A	ANGPTL4	AP2S1	ARFIP2	ARL3
ABCA3	ACTN1	ADRB3	AKAP2	ANGPTL7	AP3B1	ARHGAP1	ARL4D
ABCB7	ACTN2	ADRM1	AKAP5	ANK1	AP3B2	ARHGAP1	ARL6IP1
ABCC1	ACTN3	AEBP2	AKAP6	ANK2	AP3D1	0	ARL8B
ABCC2	ACTN4	AEN	AKAP7	ANK3	AP3M1	ARHGAP1	ARMC1
ABCC4	ACTR1B	AES	AKAP8	ANKFY1	AP3S1	1A	ARMC10
ABCD1	ACTR2	AFAP1	AKAP8L	ANKHD1	APAF1	ARHGAP1	ARMC6
ABCD3	ACTR3	AFARP1	AKAP9	ANKRA2	APBA1	5	ARMC8
ABCE1	ACTR3B	AFDN	AKIRIN1	ANKRD11	APBA2	ARHGAP1	ARMCX1
ABCF2	ACTR6	AFG3L2	AKT1	ANKRD17	APBA3	7	ARMCX3
ABCF3	ACTRT1	AGAP1	AKT2	ANKRD24	APBB1	ARHGAP2	ARNT2
ABCG2	ACVR1	AGBL5	AKTIP	ANKRD27	APBB1IP	1	ARNTL
ABCG4	ACVR2A	AGER	ALAS1	ANKRD28	APBB2	ARHGAP2	ARNTL2
ABCG5	ACVRL1	AGFG1	ALB	ANKRD34	APBB3	2	ARPC1A
ABHD11	ADAM10	AGGF1	ALCAM	A	APC	ARHGAP2	ARPC1B
ABHD15	ADAM15	AGO1	ALDH18A	ANKRD36	APCS	4	ARPC2
ABHD5	ADAM17	AGO2	1	BP1	APEX1	ARHGAP2	ARPC3
ABI1	ADAM20	AGO3	ALDH3A2	ANKRD46	APH1A	6	ARPC4
ABI2	ADAM22	AGO4	ALDH6A1	ANKRD50	APIP	ARHGAP3	ARPC5
ABI3	ADAM9	AGPS	ALDH7A1	ANKRD55	APLP1	1	ARPC5L
ABI3BP	ADAMTS	AGR3	ALDH8A1	ANKS1A	APLP2	ARHGAP3	ARR3
ABL1	12	AGRN	ALDOA	ANKS1B	APOA1	2	ARRB1
ABL2	ADAMTSL	AGTPBP1	ALDOB	ANKS4B	APOA4	ARHGAP3	ARRB2
ABLIM1	4	AGTR1	ALG8	ANKS6	APOB	3	ARRDC3
ABLIM3	ADAP1	AGTRAP	ALKBH3	ANLN	APOBEC3	ARHGAP3	ARVCF
ABR	ADAP2	AGXT	ALKBH8	ANO3	B	9	ARX
ABT1	ADAR	AHCTF1	ALOX5	ANO5	APOBEC3	ARHGAP4	ASAP1
ACACA	ADARB2	AHDC1	ALPP	ANO9	G	4	ASAP2
ACAD8	ADCK5	AHI1	ALS2	ANOS1	APOC3	ARHGDIA	ASB10
ACAD9	ADCY3	AHNAK	ALS2CR1	ANP32A	APOE	ARHGDIB	ASB13
ACADVL	ADCYAP1	AHNAK2	1	ANP32B	APOH	ARHGEF1	ASB16
ACAP1	ADCYAP1	AHSA1	ALS2CR1	ANTXR1	APOL2	1	ASCC1
ACAT2	R1	AHSG	2	ANTXR2	APOL3	ARHGEF1	ASCC2
ACBD5	ADD1	AICDA	ALYREF	ANXA1	APOL5	2	ASF1A
ACD	ADD3	AIDA	AMBRA1	ANXA10	APP	ARHGEF1	ASGR2
ACE2	ADGRA1	AIFM1	AMER1	ANXA11	APPBP2	7	ASH2L

ASIC1
ASIC2
ASIC4
ASNA1
ASNS
ASPH
ASRGL1
ASTE1
ASXL1
ATAD1
ATAD2B
ATAD3A
ATCAY
ATF2
ATF4
ATF5
ATF6
ATF7
ATF7IP
ATG10
ATG12
ATG3
ATG4A
ATG4C
ATG5
ATG7
ATG9A
ATIC
ATL2
ATL3
ATM
ATN1
ATP12A
ATP13A2
ATP1A1
ATP1A2
ATP1A3
ATP1B1
ATP2A2
ATP2B1
ATP2B2
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ATP5B
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ATP5G1
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ATP5I
ATP5J2
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ATP6V0C
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ATP6V1B
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ATP6V1H
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ATPIF1
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AURKB
AURKC
AVPI1
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BCL9L
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BEST4
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BEX1
BEX2
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BIN3
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C19orf57
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CFHR4
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CFL2
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CHEK2
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CHRNA7
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CHURC1
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CIART
CIB1
CIB3
CIC
CIDEA
CIRBP
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CIT
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CKAP5
CKB
CKS1B
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CLEC4M
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CLK3
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CLSTN1
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CLTB
CLTC
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CLUH
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CMBL
CMIP
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CMTM5
CMTM7
CMTM8
CMTR1
CMYA5
CNBP
CNDP2
CNGA3
CNGB1
CNIH2
CNIH3
CNIH4
CNKSR1
CNKSR2
CNN3
CNNM3
CNOT1
CNOT7
CNP
CNPY2
CNR1
CNST
CNTF
CNTFR
CNTLN
CNTN1
CNTN2
CNTN3
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CNTNAP2
CNTNAP4
CNTRL
CNTROB
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COG4
COG5
COG6
COG7
COIL
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COL18A1
COL1A2
COL27A1
COL4A3B
P
COL4A5
COL6A2
COL7A1
COL9A2
COL9A3
COLGALT
1
COLQ
COMMD
1
COMMD
3-BMI1
COMT
COPA
COPB1
COPB2
COPE
COPG1
COPG2
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